ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL EYE INSTITUTE

FISCAL YEAR 1972

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

U.S. NATIONAL EYE INSTITUTE

ANNUAL REPORT of program activities

July 1, 1971 *through* June 30, 1972

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STATEMENT OF THE INSTITUTE DIRECTOR
Carl Kupfer, M.D.

During the past year, the National Eye Institute has completed recruitment of key personnel in both collaborative research and program planning. As a result, consultants from the vision research community and NEI staff have developed a special initiative program in glaucoma to begin applying laboratory knowledge to clinical problems. This collaborative effort will be extended in the future to retinal disease, corneal disease and cataract. After two and one-half years, the NEI is now a fully constituted, free-standing Institute and is in a position to embark upon a major expansion and strengthening of vision research.

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1971 - June 31, 1972

REPORT OF THE ACTING DIRECTOR OF INTRAMURAL RESEARCH

Carl Kupfer, M.D.

The preceeding year has been noteworthy in the recruitment of Dr. Jin Kinoshita as Chief of the Laboratory of Vision Research. Dr. Kinoshita has been joined by Dr. Toichiro Kuwabara, Head of the Section of Experimental Pathology. With additional investigators being recruited to the already strong core of scientists, the Laboratory of Vision Research has become a national resource for vision research throughout the United States. Attention will now be turned to the Clinical Branch where the appointment of a Clinical Director is of key importance in developing a program of clinical research.

INTRAMURAL RESEARCH

Clinical Branch

Serial No. NEI(I)-71 CB 030(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Parameters of Intraocular Pressure

Previous Serial Number: Same

Principal Investigators: Carl Kupfer, M.D.
Douglas Gaasterland, M.D.

Other Investigators: Karyn Ross
Lessie McCain

Cooperating Units: Normal Volunteer Office
Pharmaceutical Development Service, CC, NIH
Biomedical Engineering, Instrumentation Branch, NIH

Man Years:

Total:	3.2
Professional:	2.7
Other:	0.5

Project Description:

Objectives: The objectives of the Glaucoma Laboratory were outlined in the annual report for FY 1971. During FY 1972 it has been possible to get a second clinical research laboratory operative, allowing expansion of the numbers of patients and volunteers being studied. A meaningful body of data related to normal volunteers in the 50 or more years-old group has started to accumulate. Continued attention has been directed toward the effects of medications upon the eight parameters of intraocular pressure in the young, normal group; similar studies have started in the older group.

Methods Employed: Eight parameters--intraocular pressure, episcleral venous pressure, total facility of outflow, true facility, pseudofacility, aqueous humor flow, P_k of Goldmann and the ocular rigidity are examined before and after medication is given topically to one eye. Replicate measurements with sophisticated subjects are made. This laboratory is still the only one where a complete study of the aqueous humor dynamics can be carried out.

Major Findings: Norepinephrine, an almost pure alpha adrenergic stimulator, causes a decrease of pseudofacility, and an increase of true facility and aqueous flow in young normal subjects. Isoproterenol, an almost pure beta

adrenergic stimulator, does not affect true facility or pseudofacility but causes a decrease of aqueous flow. The simultaneous administration of norepinephrine plus isoproterenol produces changes in the parameters of intraocular pressure closely resembling the changes induced by epinephrine, which is an alpha and beta adrenergic stimulator. The effect of epinephrine on pseudofacility is due to alpha adrenergic stimulation.

The effect of isoproterenol upon the parameters of intraocular pressure closely resembles the previously observed effect of acetazolamide. When these two medications are given simultaneously, however, the effects are additive.

Parameters of intraocular pressure in selected older normal volunteers are similar to those found in the selected group of young normal volunteers. The major difference is that the rate of flow of aqueous humor and the true facility are significantly lower.

Significance to Biomedical Research and the Program of the Institute: Study of the patterns of alteration of the parameters of intraocular pressure allows a clearer interpretation of the nature of the mechanism of action of the pharmacologic agents used to treat glaucoma. This will allow definition of the desirable and undesirable properties of various agents, and hopefully development of agents having only desirable properties.

Proposed Course of the Project: This project will continue, and will be extended in the numbers of volunteers and glaucomatous patients being studied.

Honors and Awards: Sanford R. Gifford Memorial Lecturer, 1972.

Publications:

Kupfer, C. and Ross, K.: The development of outflow facility in human eyes. Invest. Ophthal. 10: 513-517, 1971

Kupfer, C. and Ross, K.: Studies of aqueous humor dynamics in man. I. Measurements in young normal subjects. Invest. Ophthal. 10: 518-522, 1971

Kupfer, C., Gaasterland, D., and Ross, K.: Studies of aqueous humor dynamics in man. II. Measurements in young normal subjects using acetazolamide and l-epinephrine. Invest. Ophthal. 10: 523-533, 1971

Serial No. NEI(CB)-72-108
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Choriodal-Retinal Degenerative Diseases

Previous Serial Number: None

Principal Investigator: Donald R. Bergsma, M.D.

Other Investigators: Mitchel L. Wolf, M.D.
Helga Kolb, Ph.D.

Cooperating Units:
1. NEI(I)-71 CB 006(c)
2. Walter Reed Army Institute of Research, (Division of Surgery), Washington, D.C. A.R. Rosenthal, M.D. and D. Huxall, D.V.M. Study of Chloroquine Induced Damage to the Retina of the Rhesus Monkey.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The objectives of this study are to properly classify, to further clinically define, and to search for new techniques which will elucidate the cause, prevention, or therapy of selected choroidal-retinal degenerative diseases. The night blindness syndromes, familial retinal vascular disease, familial macular degeneration and effects of drugs toxic to the retina are being emphasized.

Methods Employed: Clinical studies in patients (in addition to general eye exams) consist of specialized tests of visual function (such as thresholds of visibility and color), electroretinography, and photography of the eye including fluorescein dye studies of the retinal and choroidal blood vessels. Appropriate testing of relatives is undertaken to document genetic patterns. Controlled pilot studies to evaluate Vitamin A therapy in selected diseases have began. Animal experiments have been initiated.

Major Findings: Approximately 300 patients have been studied this year on referral or recall. Although the overwhelming majority had diseases which are presently not considered treatable, most were helped by a combination of genetic counseling, discussion of prognosis, and advice regarding resources available for overcoming of handicaps imposed. Pilot studies indicate that therapeutic trials demand extremely careful and repeated baseline and followup examinations in order to document convincingly whether or not a beneficial (or toxic) effect has occurrred. Animal studies of the effect of Chloroquine on the (monkey) retina have documented early electron microscopic damage which can not be studied in the human.

Significance to Biomedical Research and the Program of the Institute: This project is being developed to bridge the gap which currently exists between our ability to detect visual damage due to choriodal-retinal diseases and our ability to properly classify, prevent and/or treat such diseases. The clinically oriented research is placing emphasis on developing and applying new diagnostic techniques and careful thereapeutic trials. The laboratory oriented research is currently being directed towards animal experimentation which can not be undertaken in humans.

Proposed Course of Project: Plans are actively being developed for expansion of the number of diagnostic tests available with particular emphasis on modifications required to properly test retinal and choroidal function in infants and children. These are necessary for more accurate classification of the diseases being studied. The addition of a technician who will be trained to administer certain prolonged but repetitive tests (for example, dark adaptation studies) will enable us to perform statistically significant trials of selected therapeutic agents. Coordination with pathologists, biochemists, geneticists, biostatistions and other basic research groups is currently being developed so that combined efforts with these disciplines can be brought to bear on the search for specific etiology and treatment of the diseases being investigated. Pilot animal studies will be expanded.

Honors and Awards: None

Publications: None

Serial No. NEI(CB)-72-109
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Ophthalmic Familial and Genetic Diseases

Previous Serial Number: None

Principal Investigator: Donald R. Bergsma, M.D.

Other Investigators: Mitchel Wolf, M.D.

Cooperating Units: NEI(I)-71 CB 006(c)

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: The objective of this study is to properly classify, to clinically define, and to search for new techniques which will elucidate the cause, prevention and/or treatment of genetic diseases affecting the eye. Please refer to the detailed description of related project, NEI(CB)-72-108, which documents Objectives, Methods and Significance concerning investigation of choroidal-retinal diseases, many of which are genetic. This project involves a broader range of ophthalmic and systemic manifications of genetic diseases.

Methods Employed: Presently a limited number of patients representing a wide variety of genetic diseases are being studied by appropriate specialized ophthalmologic and general medical techniques. Investigations are currently tailored to each situation with heavy emphasis on family studies, treatment with accepted methods when available, and genetic counseling.

Major Findings: Approximately one-hundred patients with genetic diseases involving the eye (excluding the larger group being studied primarily for choroidal-retinal degenerative diseases under project NEI(CB)-72-108) were seen on referral or recall during the current year. No general finding is apparent yet except that the majority of patients and relatives benefit from a combination of specific treatment when available, genetic counseling, and advice regarding rehabilitation and social services available.

Significance to Biomedical Research and the Program of the Institute: This project is being developed to encompass a wide variety of diseases affecting the eye because they share the common denominator of being genetically determined. Therefore certain investigative approaches involving family studies genetic counseling, statistical analysis, biochemical testing, etc. can be organized effectively to diagnose, treat, study and prevent a large number of unrelated diseases which destroy vision in apparently unrelated ways.

Proposed Course of Project: We are currently defining sub-areas within this extremely broad project. These sub-areas will then be investigated in the organized manner exemplified by the discussion in project NEI(CB)-72-108. The technical help, care, facilities, collaborative efforts, refinement of classification, development of instrumentation, controlled therapeutic trials (be they medical or surgical) and collaborative efforts described in the other project will be brought to bear on a limited number of ophthalmic-genetic diseases.

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 CB 006(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Design and Construction of Ophthalmic Instruments; Research in Methods of Evaluating Visual Processes.

Previous Serial Number: Same

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: Peter Gouras, M.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description:

Objectives: Broad objectives include the application of current procedures for psychophysical tests, improving their form and scope and enhancing the usefulness of any ophthalmic instruments.

Since most threshold measurements are necessarily subjective, one of our aims is to replace or confirm subjective data by an approach to objectivity.

Methods Employed: In collaboration with clinical associates, routine psychophysical and other ophthalmic tests are conducted on appropriate patients. Findings are reported, discussed and entered in the medical records.

There has also been a mutually beneficial exchange of ideas, instrumentation and assistance with research associates in the field of electrophysiology of the eye.

Major Findings: Psychophysical tests were done on about 310 patients during the year in diagnostic or follow-up studies of retinal degenerations of whatever origin.

The Eye-Trac machine has been further modified for monitoring various types of eye movements and approximately 78 recordings have been made.

Of particular interest was a series of recordings from a patient with congenital nystagmus before, during and after medication with phenobarbital.

Many small instruments or parts have been designed and constructed for use in perfusion of eyes, electrophysiological studies, photography, laser coagulation, glaucoma studies and other clinical and laboratory procedures.

Significance to Biomedical Research and the Program of the Institute: While the amount of independent research possible has been quite limited, it is felt that contributions to related clinical and research programs have been significant.

Proposed Course of Project: It is expected that current studies will be continued and hopefully extended to include assistance in monochromatic fundus photography and binocular fluorescein angiography.

Honors and Awards: None

Publications:

Gouras, P., Carr, R.E., and Gunkel, R.D.: Retinitis Pigmentosa in abetalipoproteinemia: Effects of Vitamin A. Invest. Ophthal. 10: 784-793, 1971

Serial No. NEI(I)-71 CB 024(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cystinosis

Previous Serial Number: Same

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Joseph Schulman, M.D.
J. Edwin Seegmiller, M.D.

Cooperating Units: Laboratory of Human Genetics, NIAMD

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

This project is being terminated.

Serial No. NEI(I)-71 CB 031(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Lymphocyte Transformation in Sympathetic Ophthalmia

Previous Serial Number: Same

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Richard Anderson, B.S.
Paul O'Brien, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

This project is being terminated.

Honors and Awards: None

Publications:

Wong, V.G., Anderson, R., and O'Brien, P.: Sympathetic ophthalmia and lymphocyte transformation. Amer. J. Ophthal. 72: 960-966, 1971

Serial No. NEI(I)-71 CB 032(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Lattice Corneal Dystrophy

Previous Serial Number: Same

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: R.A. Delellis, M.D.
G.G. Glenner, M.D.

Cooperating Units: NIAMD

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

This project is being terminated.

Serial No. NEI(CB)-72-115
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Experimental Ocular Histoplasmosis

Previous Serial Number: None

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Richard Anderson

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	1.1
Other:	0.0

Project Description:

Objectives: To create an experimental model for presumed ocular histoplasmosis in man.

Methods Employed: Albino rabbits were infected systemically with yeast and myceliol phases of histoplasma capsulatum. Infective inoculum ranged from 5 to 40 M(million) organisms.

Major Findings: Animals infected with yeast cells developed bilateral endophthalmitis within 10 days of infection. The severity of the ocular changes was proportional to the dose. In those animals which received myceliol forms of the organism, focal choroidal pathology represented by punched-out changes in the fundus were noted. Endophthalmitis did not occur in any of these animals. It appears at this time of our study that following the initial ocular insult no further lesions could be produced on reinfection.

Significance to Biomedical Research and the Program of the Institute: The etiology of presumed ocular histoplasmosis in man is unknown. This represents the first reproducible experimental animal model in which the ocular lesions produced are characteristically similar in appearance to those observed in man.

Proposed Course of Project: To be terminated.

Honors and Awards: None

Publications: None

Laboratory of Vision Research

ANNUAL REPORT
LABORATORY OF VISION RESEARCH
July 1, 1971 - June 30, 1972

by Jin H. Kinoshita
Chief, Laboratory of Vision Research

During the past year the major change to occur in the Laboratory of Vision Research was the addition of twelve new investigators and technicians to the research staff. The new personnel added to the group already active at the Institute constitute an impressive array of scientists who are devoted to vision research. The new research staff blends the old and the young, the basic and the clinical interests and the various fields of medical and biological disciplines.

In restructuring the Laboratory, four sections were created:

1. Experimental Embryology, headed by Dr. Alfred J. Coulombre

2. Experimental Pathology, headed by Dr. Toichiro Kuwabara

3. Neurophysiology, headed by Dr. Peter Gouras

4. Biochemistry, led by Dr. Jin H. Kinoshita, serving as Acting Head

The research staff covers a broad field of interests as revealed in the individual progress reports and the contemplated studies proposed by the new investigators. In essence, great emphasis is placed on projects that will lead to an understanding of a disease process or how an eye organ functions. During the past year progress has been made in uncovering the complex nature of the chemistry of the visual pigment, rhodopsin. The de-novo synthesis of rhodopsin in isolated retina has been examined. The essential need of phospholipids in the regeneration of rhodopsin in the dark has been demonstrated. The complex processing of visual information in the retina as well as in the visual cortex has been extensively studied. The detailed investigation of one type of retinitis pigmentosa has led to a possible breakthrough in the treatment of this type of retinal dystrophy. Studies dealing with the factors that control the development of the eye are actively pursued with the hope that insights into the congenital eye defects may be gained.

Another noteworthy addition to the Eye Institute is the acquisition of new laboratory space. To provide for the expansion of the research staff, additional space has been made available in the remodeled laboratories of Building 6. Although some of the activities of the Laboratory of Vision Research will remain in Buildings 9 and 10, most of the basic research personnel will be housed in the new quarters in Building 6.

NEW INVESTIGATORS IN THE LABORATORY OF VISION RESEARCH

NAME	PREVIOUS POSITION	PROJECTS TO BE PURSUED AT THE EYE INSTITUTE
BIOCHEMISTRY SECTION		
I. Jin H. Kinoshita, Ph.D.	Professor of Biochemical Ophthalmology, Harvard Medical School	1. Development and prevention of sugar cataracts. 2. Congenital cataracts in mice. 3. Lens proteins in cataract formation.
A.) S.D. Varma, Ph.D.	Visiting Scientist, Oakland University	Sugar cataracts
B.) I. Kabasawa, M.D.	Research Associate, Harvard Medical School and Juntendo University, Japan	Lens glycoproteins
C.) H. Obazawa, M.D.	Research Associate, Harvard Medical School and Assistant Professor, Keio University, Japan	Congenital cataracts
D). L.O. Merola, M.S.	Associate, Harvard Medical School	Cataracts
II. Helen Hess, M.D.	Associate Professor of Neuropathology, Harvard Medical School	Biochemical composition of photo-receptors, neuronal and glial membranes in normal animals and in rats with retinitis pigmentosa.
A.) J. Derr, A.B.		

NAME	PREVIOUS POSITION	PROJECTS TO BE PURSUED AT THE EYE INSTITUTE
III. Gerald Chader, Ph.D.	Instructor in Ophthalmic Research, Harvard Medical School	1. Cyclic AMP and its associated enzymes in the retina. 2. Retinal dystrophies in rats. 3. Retinoblastomas 4. Steroid binding in the retina.
A.) J. Kostas, A.B.		
EXPERIMENTAL PATHOLOGY SECTION		
IV. Toichiro Kuwabara, M.D.	Professor of Pathology, Harvard Medical School	1. Light damage effects on retina. 2. Laser effects on retina. 3. Pathology of glaucoma.
A.) V. Lauderdale, M.S.		
B.) D. Player, B.S.		

Serial No. NEI(I)-71 LVR 001
1. Laboratory of Vision Research
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Ocular Morphogenesis

Previous Serial Number: Same

Principal Investigator: A.J. Coulombre, Ph.D.

Other Investigators: Jane L. Coulombre, B.S.
D. Newsome, M.D.
K. Kenyon, M.D.
D. Reese, Ph.D. (NIH Postdoctoral Fellow)

Cooperating Units: Departments of Ophthalmology and Obstetrics-Gynecology and The Genetics Clinic, The Johns Hopkins Hospital, Baltimore, Maryland

Man Years:

Total:	4.92
Professional:	4.92
Other:	0.00

Project Description:

Objectives: We seek to identify and to characterize the tissue interactions that control the orderly growth of the vertebrate eye. Most congenital defects of the eye are attributable to interference with these interactions.

Methods Employed: Routine chemical, histochemical, experimental embryologic, light microscopic (including phase microscopy of living cells) electron-microscopic, pharmacologic, tissue culture and autoradiographic techniques are used to analyze the development of the visual system of the domestic fowl. This year we adapted existing experimental embryologic and tissue culture techniques and assembled them into a procedural sequence that permits the identification of "initiator layers" (vide infra) produced transiently by epithelia during embryonic development. This procedure opens the way for the identification and analysis of these recently discovered layers, which appear to mediate some inductive interactions among tissues. Newts and embryos and fetuses of frogs, domestic fowl, rabbits, mice and man were used in our investigations.

Major Findings: During FY72 new information was obtained or published on:

I. Cornea: The following findings relate to the role of collagen and other glycoproteins in the development of the physical and optical properties of the cornea and to the identification of the determinants of corneal transparency: A. Cornea of the domestic fowl: 1. The stroma of the developing cornea is initially deposited by the anterior corneal epithelium between the third and the tenth days of incubation, as a cell-free primary stroma containing an orthogonal ply of collagen fibrils. 2. The Golgi apparatuses of the basal epithelial cells are involved in processing the collagen deposited in the primary stroma. 3. The innermost layers of this ply are deposited between the third and the fifth days of incubation. The two axes of the orthogonal ply are oriented dorso-ventrally and naso-temporally and are in register from layer to layer in the ply. 4. The outermost layers of the collagenous matrix are deposited in sequence beneath the corneal epithelium between the fifth and the tenth days of incubation and have orthogonal axes that rotate between 1° and 2° from layer to layer, a rotation that is in the same direction in both eyes, and therefore, assymetric about the body midplane. 5. The primary stroma swells quickly on the fifth day of incubation and is invaded from the margin by fibroblasts. This swelling may be a necessary condition for fibroblastic invasion and for the conversion of the primary stroma into a mature secondary stroma. 6. Transient interruption of collagen excretion into the primary stroma with L-azetidine-2-carboxylic acid produces a collagen-sparse lesion. When excretion recommences, the collagen is once again deposited in a regular orthogonal ply and the incremental rotation of the axes from layer to layer is resumed. The results indicate that the factors that determine the collagenous architecture do not include self-assembly on previously deposited layers of collagen. B. Frog cornea: 1. The tadpole eye has an inner cornea containing cells and strap-like bundles of collagen fibrils, and an outer cornea with a cell-free stroma containing an orthogonal matrix of collagenous layers. The stroma of the inner cornea resembles that of mammals. The stroma of the outer cornea resembles the outer portion of the corneal stroma of the domestic fowl, especially in the incremental rotation in successive layers of the axes of the orthogonal collagenous grid. The two corneas fuse, in the tadpole, at metamorphosis. 2. The collagen fibrils of the outer cornea are larger in diameter than those of the inner cornea. The results indicate that several strategies are in use among vertebrates for construction of a transparent cornea, open the way for an examination of factors common to all strategies and afford us another way to identify the determinants of stromal geometry. C. Rabbit cornea: (in collaboration with Wilmer Institute, Johns Hopkins Hospital): Retrocorneal membranes produced by repeatedly freezing the cornea contain collagen and collagen-excreting cells that may be of endothelial origin. D. Human cornea: 1. (in collaboration with Genetics Clinic, Johns Hopkins Hospital): In the inherited disorders of acid mucopolysaccharide metabolism, corneal clouding is caused by accumulations of storage material both within and around the stromal keratocytes. Treatment of affected patients by plasma infusions results in a reversal of the storage phenomenon as can be detected by electron microscopy. 2. (with the cooperation of Department of Obstetrics and Gynecology, Johns Hopkins Hospital): A

descriptive ultrastructural study of the development of the human, fetal cornea deals primarily with the morphogenesis of the corneal stroma and particularly the development of the highly organized architecture of the collagenous lamellae.

II. Lens: Previous work established that complete removal of the lens from the eye of the newt is followed by metaplastic transformation of the dorsal iris into a normal lens. The earliest detected step in this metaplastic change is an increase in the synthesis of ribosomal ribonucleic acid (rRNA). When the dorsal iris is isolated in tissue culture changes occur in rRNA synthesis that are quantitatively, qualitatively and temporally equivalent to those occurring in the lenseless eye, save that a new lens has not yet been developed under these conditions. On the basis of these previous findings studies are in progress to identify the factors that initiate the enhanced synthesis of rRNA and to find conditions that allow complete transformation of iris cells into lens cells in culture.

III. Retina: A. Neural retina: 1. A technique developed by the Section in FY71 was used to establish that the first ganglion cell axons appear in the neural retina of the chick embryo at about two and one half days of incubation, immediately grow toward the embryonic fissure and form fascicles which anastamose and which increase in diameter and in concentration as the embryonic fissure is approached. 2. By five days of incubation two superimposed sets of fascicles are evident and the two sets enter the fissure at different angles. 3. Beginning at five days of incubation an arcuate pattern is formed by the fascicles in the dorso-nasal quadrant of the retina. Fiber bundles become sparse at the center of the pattern. It seems probable that the arcuate area, which disappears late in development, is an abortive foveal region (no fovea exists in the adult of this species). 4. Circumferential bundles of ganglion cell fibers were discovered around the outer edge of the nerve fiber layer; these enter the outermost end of the embryonic fissure. It is not known presently whether this tract differs functionally from the remainder of the nerve fiber layer. 5. The point from which the circumferential fibers diverge originates on the temporal side and migrates about 90° nasal as development proceeds. 6. Centrifugal fibers, presumably from cells in the isthmo-optic nucleus, were first detected at seventeen days of incubation. These results are the first description of the development of the nerve fiber layer viewed intact, and set limits to the types of hypotheses that can be entertained concerning axonal guidance during embryogenesis. B. Pigmented epithelium: 1. Cell birthdays: Cells of the pigmented epithelium undergo their final cell divisions at times specific for each region of this layer. Cell division ceases earlier in the fundus than at the equator and continues longest at the edge of the optic cup. 2. Explants of pieces of pigmented epithelium and cloned cell cultures derived from single pigmented epithelial cells produce and excrete collagen. The collagen aggregates into fibrils with a macroperiod of from 530 to 600 Å. Pigmented epithelium from older embryos produced fibrils of larger diameters than those from younger embryos. These findings strengthen the presumption that collagen-containing acellular layers which appear transiently during development next to the embryonic pigmented epithelium may be

produced, at least in part, by that epithelium. The findings also indicate that fibrocytes are not the only source of collagen (as has long been widely held), but that some epithelial cells, at least early in their development, can produce and excrete collagen.

IV. Sclera. A. Cartilagineous sclera: 1. Previous work in this Section and at other institutions established that the hyaline cartilage of the sclera is induced by the pigmented epithelium. Work in FY72 in this Section has established that this potency of the pigmented epithelium exists before eight or nine days of incubation but not thereafter. This information not only establishes the transiency of the potency, but also facilitates the search for the physical basis of such potency. 2. Cell-free products excreted by cultured pigmented epithelial cells have been demonstrated to be potent to initiate or permit the differentiation of compentent cells to form chondrocytes. This observation raises the possibility that some developmental inductive interactions may be mediated by cell free products ("initiator layers") of the inducing tissue. B. Scleral ossicles: Each scleral ossicle is induced in the embryonic perilimbic mesenchyme by a transient conjunctival papilla. Collagen-containing deposits beneath the papillae seem to mediate this induction. In FY71 we reported that L-azetidine-2-carboxylic acid, a proline analog which inhibits collagen excretion, prevents development of scleral ossicles. This year we established the following points: 1. Ossicle differentiation is sensitive to the analog only from the end of the fifth to the end of the seventh days of incubation and not before or after that period. 2. The conjunctival papillae mature late and abnormally when the analog is administered. 3. These effects are stereoisomerically specific since D-azetidine-2-carboxylic acid produces no such effects.

Significance to Biomedical Research and the Program of the Institute: Identification and characterization of the tissue interactions that control ocular development is indispensable to establishing the etiology of most congenital eye defects. Particular attention is currently focused on newly-discovered, cell-free, collagen-containing "initiator layers" that are transiently produced by several epithelia of the embryonic eye. These layers have been demonstrated, in some cases, to support specific types of cell differentiation and to determine the three-dimensional architecture of the mesenchymally derived tissues that develop within them. It appears possible that "initiator layers" are importantly involved in the differentiation and morphogenesis of most organs of the body.

Honors and Awards:

Distinguished Scientist Lecturer, Tulane University, March 20, 1972

Publications:

Goldberg, S.: 197 - A pyridine-silver method for staining whole mounts of embryonic retina, optic tract and optic tectum. Stain Techn. (in press)

Karlsberg, R.M., Jared, M., Emery, W., Green, M., Valdes-Depena, M., and Coulombre, A.: Iris and chamber angle anomalies in a child with multiple congenital anomalies. Arch. Ophthal. 86: 287-292, 1971

Trelstad, R., and Coulombre, A.: Morphogenesis of the collagenous stroma in the chick cornea. J. Cell Biol. 50: 840-853, 1971

Coulombre, J.L., and Coulombre, A.: Metaplastic induction of scales and feathers in the corneal anterior epithelium of the chick embryo. Develop. Biol. 25: 464-478, 1971

Coulombre, A.J.: Eye (vertebrate). In Lapedes, D.N., (Ed.): McGraw-Hill Encyclopedia of Science and Technology, Vol. 5, Third edition. New York, McGraw-Hill Book Co., 1971, pp. 176-185.

Goldberg, S. and Coulombre, A.: Topographical development of the ganglion cell fiber layer in the chick retina. A whole mount study. J. Comp. Neurol. (in press)

Coulombre, A. and Coulombre, J.: Corneal development. IV. Interruption of collagen excretion into the primary stroma of the cornea with L-azetidine-2-carboxylic acid. Develop. Biol. (in press)

Newsome, D.: Cartilage induction by retinal pigmented epithelium of chick embryo. Develop. Biol. (in press)

Goldberg, S.: Silver staining featuring rapid reduction for whole mounts of retina and optic pathways in chick embryos. Stain Tech. 47: 65-69, 1972

Serial No. NEI(LVR)-72-111
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Chemistry of the Cornea

Previous Serial Number: None

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To isolate tissue-specific soluble proteins from the epithelium and stroma of the cornea and to characterize these macromolecules by physical, chemical and immunological techniques.

Methods Employed: Distinct proteins will be isolated and fractionated from pooled calf corneas by use of pressure chromatography on columns of glass beads coupled with ion-exchange chromatography. The purity of individual fractions will be determined by the number of bands obtained by staining following polyacrylamide gel electrophoresis.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Successful isolation of a tissue-specific soluble protein from the cornea in large amounts would provide source material for immunological studies to determine whether the macromolecule functions as a transplantation antigen in experimental animals. If such studies were successful, further investigations with a chemically modified protein might suggest an approach for control of corneal graft rejection in humans.

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 LVR 010(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972.

Project Title: Chemistry of the Vitreous Body

Previous Serial Number: Same

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: This project is being terminated this year. The methods developed in this research work will be incorporated into the new study on corneal proteins. Sialoproteins and sialoglycans will be investigated in the latter project when it is deemed appropriate.

Serial No. NEI(I)-71 LVR 027(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Induction of Buphthalmos in Chicks by Feeding a High Level of Glycine

Previous Serial Number: Same

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: Max Rubin, Ph.D. (University of Maryland)
Douglas Gaasterland, M.D.

Cooperating Units: Department of Poultry Science, University of Maryland

Man Years:

Total:	0.9
Professional:	0.7
Other:	0.2

Project Description:

Objectives: To study the chemical and physical factors which control the size and shape of the vitreous during development of the eye as well as maturity.

Methods Employed: Weight determinations were made on the total eye and various ocular tissues. Colorimetry was employed to measure the quantity of each of the major macromolecules in dialyzed chicken vitreous.

Major Findings: The feeding of 8% glycine to newly hatched chicks raised for a period of 7 weeks on a basal diet supplemented with 5% gelatin and 10mg % nicotinic acid produces a depression in body growth and an enlargement of the eyeball (buphthalmia). Elevation of the level of gelatin from 5 to 8% and reduction of glycine from 8 to 6% in the diet resulted in elimination of the growth depression for the male but not for female chicks. The eye effect was retained in both sexes with the change in dietary regimen. The diet at the lower glycine level was judged to be adequate for all known nutrients and buphthalmia could not be prevented by additional supplements of vitamin B_{12} or folic acid as reported in the literature. Supplementation with arginine and excessive amounts of niacin were also without effect. The increase in eye size in these birds was due in large part to an increase in the size of the vitreous and the severity of the syndrome was found to be sex-dependent.

Glycine-fed females exhibited a 30% reduction in the protein concentration as well as the total content of soluble proteins in the vitreous when compared to controls of the same sex. These parameters remained essentially unaltered in males fed the amino acid-supplemented diet. No change was observed with glycine-fed birds of either sex in hydroxyproline level over controls whether expressed in terms of total amount in the tissue or per gram wet vitreous. Males however exhibited a three-fold increase over females on either parameter.

Significance to Biomedical Research and the Program of the Institute: Chicks grown on a high-glycine diet represent the first nutritional model for the study of buphthalmos in experimental animals. Since chickens possess a deficient blood-brain barrier during the first month post-hatching, buphthalmic animals prove not only to be useful as tools for studying biochemical changes which take place in developing vitreous but in the maturing nervous system as well.

Proposed Course of Project: Glycine has been postulated to function as an inhibitory neurotransmitter at certain sites in the central nervous system. Feeding studies will be conducted with a more well-defined inhibitor, namely γ-aminobutyric acid and the corresponding excitatory neurotransmitter L-glutamic acid for comparison with glycine to ascertain their respective abilities either to induce or block the buphthalmic syndrome. The investigation of the ability of various glycine-rich pepetides and proteins to substitute for gelatin in the diet will be resumed with a new appreciation of the differential utilization of free glycine by the sexes.

Honors and Awards: None

Publications:

Rubin, M. and Helmsen, R.J.: Differentiation of growth and buphthalmos syndromes in chicks fed high levels of crystalline glycine. Poult. Sci. (abstract, in press).

Serial No. NEI(I)-71 LVR 009(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Physical Chemistry of Model Gel Systems

Previous Serial Number: Same

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: Richard Shrager, Physical Sciences Lab., DCRT
Dr. Harold Edelhoch and Dr. Pieter van Jaarsveld
Clinical Endocrinology Branch, NIAMD

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: To study the physical and chemical parameters of model systems which are pertinent for transparency and opacity of gel systems or which may in any way be of significance to the biochemistry of vision.

Methods Employed: The principal method used in these studies has been analytical ultracentrifugation, since it has been demonstrated to be the most effective technique for studying systems of interacting macromolecules. Considerable emphasis has also been given to the development of sophisticated computer techniques for data reduction and analysis of sytems of this type.

Major Findings: Progress has been made in studies on the binding of retinol binding protein (RBP) to prealbumin (PA). By measuring the concentration distributions of RBP and PA alone and in various mixtures in the ultracentrifuge using scans at different wavelengths in order to differentiate between the two proteins, it appears possible to calculate the extent of RBP binding to PA as a function of free RBP concentration. Four binding sites per PA molecule appear to be involved, and the association constants are being determined. Further progress has been made in computer techniques for the analysis of problems of this type and for other macromolecular associations. The utility of the different types of measurable average molecular weights has been explored, and it has been found that while the weight-average is

the only average of significance for monomer-n-mer systems, both the weight- and the number-average molecular weights are important in the analysis of discreet systems such as a monomer-dimer-tetramer.

Significance to Biomedical Research and the Program of the Institute: The techniques of analysis of interacting systems which have been developed are of importance to a wide variety of biological problems besides those of immediate interest here such as the work on RBP binding to PA, the association of detergents with visual pigments, or associations involving phospholipids and visual pigments. The studies on RBP binding to PA are of significance because this is the major physiological mechanism which is involved in the plasma transport of retinol which is involved in the function of rhodopsin.

Proposed Course of Project: Major emphasis will be given to the completion of the study on the binding of RBP to PA. The computational techniques which have been developed will be applied to this problem as well as to other problems of related interest.

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 LVR 008(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Chemistry of Rhodopsin

Previous Serial Number: Same

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: To study the structural and functional aspects of the bovine rhodopsin molecule.

Methods Employed: Delipidated opsin was prepared from chromatographically purified rhodopsin by repeated extraction with a chloroform-methanol mixture and with petroleum ether. This material was used for the amino acid analyses and for the spectral studies, and its molecular weight was determined by sedimentation equilibrium in organic solvents in the analytical ultracentrifuge.

Major Findings: Further significant improvements have been effected in the use of a modulatable He-Ne laser as a light source for the analytical ultracentrifuge which would be specifically suitable for rhodopsin studies since its emission at 633 nm was essentially nonbleaching. A multiplexer has been developed which makes it possible to pulse the laser when a specific cell in the rotor is in the proper position. This facilitates multi-cell runs by permitting the selection of a specific cell to be observed and avoiding the optical degradation resulting from multiple images. The short pulse duration possible with this instrument also improves image resolution in either single or multi-cell runs. The higher intensity of this light source has permitted the use of finer grained, higher resolution photographic emulsions for recording the images. This overall improvement in resolution has revealed previously undetected aberrations in the optical system of the ultracentrifuge. and considerable effort has been expended to correct these or compensate for their effects.

Delipidated opsin, like the native rhodopsin from which it is derived, is insoluble in aqueous solvents in the absence of detergents. This has led to the use of organic solvent systems which are suitable for solubilizing this apoprotein. Extensive studies have demonstrated that 1 M pyridine buffered 2-chloroethanol (CE-P) and 1 M pyridine buffered 2-bromoethanol (BE-P) were the best solvents for ultracentrifugal analyses and that hexafluoroacetone trihydrate (HFA) was the best solvent for spectral studies. The marked differences in the densities of the CE-P and BE-P solvent systems permit the simultaneous ultracentrifugal determination of both the partial specific volume and molecular weight of the delipidated opsin with good precision. A value of 33,000 has been obtained for the molecular weight of opsin in these solvent systems. The validity of this method has been demonstrated with proteins of known molecular weight. These analyses have been greatly facilitated by the use of the laser light source.

The molecular weight of a protein may also be determined from its amino acid composition. A computer program has been developed which facilitates this by optimizing the determination of integral values for the amino acid composition by a least-squares fitting procedure. Published data from this laboratory as well as that published by Heller and by Shields have been analysed by this technique. All of these analyses give molecular weights for the apoprotein lying between 31,000 and 36,000, which must be considered to be in good agreement with the ultracentrifugally determined value. Dr. Shichi has begun applying this analysis to his studies on the composition of rhodopsin peptides.

The molar extinction coefficient of delipidated opsin was calculated from the UV spectra of solutions of known concentration in HFA. By comparing the spectra of other proteins in aqueous solvents and in HFA, the shifts in wavelength and magnitude of the absorption maxima obtained with the two solvent systems have been determined. Using appropriate corrections based on this data, a hypothetical molar extinction coefficient of 77,800 at 279 nm has been calculated for delipidated opsin in aqueous solvents. By applying a 279 nm/498 nm ratio of 1.70, as obtained for the purest solutions of rhodopsin in a detergent solution, a molar extinction coefficient of 45,700 has been calculated for rhodopsin at 498 nm, a value in good agreement with those which have been obtained by several other investigators using other methods.

Significance to Biomedical Research and the Program of the Institute:
In addition to its demonstrated facilitation of ultracentrifugal analyses on rhodopsin, the multiplexed laser light source represents a major technological innovation in analytical ultracentrifugation. Several leading investigators in the field of analytical ultracentrifugation will shortly be installing multiplexed laser light sources of the type developed here, and it appears probable that this will become a widely utilized technique in this field.

It is felt that the studies reported here offer convincing evidence for the values which have been obtained for the molecular weight and molar extinction coefficients for rhodopsin, which have been the subjects of recurrent controversies for a number of years. Knowledge of these values is important

for many quantitative studies on the role of rhodopsin in the visual process. In addition, the ultracentrifugal and spectral methods are of general applicability for other lipoproteins, and the least-squares fitting technique is applicable to any biochemical problem involving amino acid analysis.

Proposed Course of Project: Continued studies will be in two principal areas. The first of these is on the effects of bleaching on the configuration of rhodopsin in solution and in the rod outer segment membrane as revealed by hydrodynamic behavior. The second is a study of the association of various detergents with rhodopsin with the intent to utilize this information to estimate the strength of the bonds involved in the incorporation of rhodopsin in the rod outer segment membrane.

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 LVR 015(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Synthesis of Sugar-Containing Polymers in Retina

Previous Serial Number: Same

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. In the process of renewal of photoreceptor outer segment disc membranes, rhodopsin, a glycoprotein, must be transported from the inner segment and incorporated into disc membranes with a specific orientation in space. This project was designed to determine where and when sugars are added to the polypeptide and what role they play in the transport and assembly of rhodopsin into disc membranes.

Methods Employed: Standard biochemical techniques were employed inluding incubation of bovine retinas, isolation of rod outer segments by density gradient centrifugation and purification of rhodopsin by column chromatography.

Major Findings: The incorporation of radioactive glucosamine appears to be a rather late event in the process of synthesis and transport of rhodopsin to the outer segment. An opsin-like molecule can be found in the outer segment at early times, an apparent precursor. However, since isolated outer segments fail to incorporate glucosamine, most of the additions of sugar residues probably take place in the inner segment.

Significance to Biomedical Research and the Program of the Institute: The glycosylation of rhodopsin appears to be similar to the glycosylation of many glycoproteins in that the sugar residues are added as an immediate prelude

to transport. Moreover, it appears that a fully glycosylated opsin is incorporated into the disc membranes prior to the addition of Vitamin A. Consequently the sugars may be necessary for each of these events to occur. A defect in this glycosylation process could be the cause of impaired renewal or function of the photoreceptor cells.

Proposed Course of Project: Studies will be centered on precise definition of the temporal relationship between polypeptide synthesis, glycosylation and appearance of the product in the outer segment. Efforts will also be directed toward study of possible control mechanisms involved in this sequence of events.

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 LVR 016(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Protein Synthesis in the Retina

Previous Serial Number: Same

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. This project was designed to elucidate some of the biochemical events involved in this process, in particular the control of rhodopsin transport to the outer segment and the site of the addition of Vitamin A to the rhodopsin polypeptide.

Methods Employed: Ordinary biochemical methods were used, including incubation of bovine retinas, isolation of outer segments by density gradient centrifugation and purification of rhodopsin by column chromatography.

Major Findings: Pulse labeling with radioactive amino acid confirmed a previous observation that the protein portion of rhodopsin was synthesized in the inner segment and transported to the outer segment as observed by others *in vivo*. The appearance of an opsin-like polypeptide in the outer segments suggested that Vitamin A is added after the opsin is transported to the outer segment. This transport process was inhibited by agents that interfere with microtubule function.

Significance to Biomedical Research and the Program of the Institute: These results verify the reliability of this *in vitro* system for the study of the renewal process. In addition it appears that Vitamin A may not be necessary for the transport of opsin and the assembly of membranes but that

microtubule function may be essential. In this respect, outer segment renewal bears some resemblance to axoplasmic flow which is essential to maintain nerve conductance. A great deal is known about axoplasmic flow and the information can be applied to the problem of outer segment renewal.

Proposed Course of Project: Efforts will be concentrated on the identification of opsin as a precursor in the outer segments, on the site of addition of Vitamin A and on the extent of the analogy between axoplasmic flow and outer segment renewal.

Honors and Awards: None

Publications:

O'Brien, P.J., Muellenberg, C.G., and Bungenberg de Jong, J.J.: Incorporation of leucine into rhodopsin in isolated bovine retina. Biochemistry 11: 64-70, 1972

Serial No. NEI(I)-71 LVR 012
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemistry of Visual Pigments

Previous Serial Number: Same

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: (1) To elucidate the chemical structure of the rod pigment rhodopsin for an understanding of the molecular mechanism of rod (dim light) vision. (2) To continue investigations on a possible function of lipid in the structure of rhodopsin.

Methods Employed: Such biochemical methods as centrifugation, column chromatography and spectroscopic analysis. High voltage electrophoresis and amino acid analysis.

Major Findings: (i) Heat stability of rhodopsin is markedly reduced after phospholipid is removed from the pigment. (ii) The addition of phospholipids to delipidated rhodopsin results in an increase in heat stability. (iii) Mg^{+2} ions increase heat stability of rhodopsin: Ca^{+2}, K^{+} and Na^{+} are without effect. (iv) After cleavage of delipidated opsin with cyanogen bromide, 9 peptides were separated on a peptide map prepared by chromatography and high voltage electrophoresis. One of the major peptides was isolated and found to contain lysine(1), arginine(1), aspartic acid(3), threonine(1), serine(2), glutamic acid(2), alanine(2), valine(1), isoleucine(1), leucine(1) and phenylalanine(1); histidine, methionine and tyrosine were absent (the number of residue shown in the parenthesis).

Significance to Biomedical Research and the Program of the Institute: The results obtained strongly support that phospholipid is essential for rhodopsin in order to maintain a preferred (native) conformation. Abnormal rod function observed under certain pathological conditions, e.g., retinal dystrophy, may be related to a decrease in stability of rhodopsin. The present finding on the phospholipid requirement for rhodopsin may be of biomedical significance in this respect.

Proposed Course of Project: (1) Characterization of the nature of interaction between rhodopsin and phospholipid. (2) Elucidation of the chemical structure of rhodopsin.

Honors and Awards: None

Publications:

Shichi, H.: Circular dichroism of bovine rhodopsin. Photochem. Photobiol. 13: 499-502, 1971

Shichi, H.: Biochemistry of Visual Pigments. II. Phospholipid requirement and opsin conformation for regeneration of bovine rhodopsin. J. Biol. Chem. 246: 6178-6182, 1971

Serial No. NEI(LVR)-72-110
1. Laboratory of Vision Research
2. Section on Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Information Processing in the Visual Cortex of the Rhesus Monkey.

Previous Serial Number: None

Principal Investigator: Bruce M. Dow, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other	0.0

Project Description:

Objectives: Evidence is accumulating from several laboratories involved in single cell neurophysiology that the visual system does not simply reproduce the details of the external world in the manner of a photograph, but instead abstracts specific features or highlights of the world for more detailed processing. Such features include contour orientation, depth in space, direction or velocity of movement, dark-light contrast and color.

The present project arose out of the discovery that neurons in one of the "association" areas of cat cerebral cortex were specifically sensitive to movements of visual objects, but showed little specificity for size, shape or position of objects. The results suggested that details of stimulus movement and details of stimulus form were being processed in parallel by different groups of cells. The work was extended to the rhesus monkey striate cortex for two main reasons. First, it seemed important to determine whether information was being processed in parallel by the cells in a primary visual cortical area, the well developed color vision of the rhesus monkey permitting the introduction of color as an additional stimulus variable. Second, it seemed evident that the study of feature detection by single brain cells should ultimately be carried out in awake, behaving animals, the rhesus monkey being considerably more suitable for such behavioral experiments than the cat.

Methods Employed: These include standard electrophysiologic techniques for recording extracellular impulses from single neurons with microelectrodes in paralyzed, anesthetized animals. Independently variable stimulus parameters include shape, size, orientation, velocity and direction of movement, color, intensity, and background illumination. Recording sites can be marked by the injection of colored dye from the microelectrode tip.

Major Findings: There are three distinct populations of cells in the foveal projection area of rhesus monkey striate cortex. Spatial opponent cells respond specifically to properly oriented moving or stationary slits of light. Responses are markedly reduced when the stimulus orientation is changed by more than 15 or 20 degrees in either direction. These cells receive synergetic input from both the "red sensitive" and "green sensitive" cone channels and show no evidence of specificity for color.

Color opponent cells, on the other hand, receive antagonistic input from different cone channels. These cells have not shown any sign of spatial inhibition, the chromatically antagonistic inputs being spatially coextensive.

Spatial color cells show orientation specificity which is dependent on stimulus color. These cells receive excitatory input from either the "red sensitive" or the "green sensitive" cone channel in a line-shaped central region of their receptive fields and inhibitory input from the other cone channel in one or both flanks. The spatial separation of color opponent inputs to these cells appears similar to what has been reported for most of the cells in the rhesus monkey lateral geniculate nucleus, suggesting that the spatial color cells represent an earlier stage in visual processing than either the spatial opponent or the color opponent cells.

The major implication of these findings is that orientation and color are largely processed in parallel by different groups of neurons in the rhesus monkey striate cortex.

Significance to Biomedical Research and the Program of the Institute: This work provides strong support to the notion that visual information processing by the brain involves feature detection rather than photographic reproduction.

Proposed Course of Project: The next step is to examine neurons in another cortical region receiving direct projections from striate cortex, to see whether further segregation of function (as was present in cat association cortex) can also be found in the monkey. Ultimately, the goal is to determine whether cells with specificities for certain stimulus features are in fact involved in the visual perception of real objects with those features. The technology for recording single neurons in awake behaving monkeys has been developed at NIH in recent years and is readily accessible. A new laboratory, utilizing this technology, is currently in the planning stages.

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 LVR 026
1. Laboratory of Vision Research
2. Section on Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Physiology of the Primate Visual System

Previous Serial Number: Same

Principal Investigator: Peter Gouras, M.D.

Other Investigator: Eleanor Collins

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.9
Other:	0.1

Project Description:

Objectives: The broad aim of this project is to understand the cellular organization underlying visual perception. We have concentrated on the visual system of the Rhesus monkey because its similarity to the human visual system allows for more direct comparisons with human perception about which there is a large volume of information.

The recent work has concentrated on the area of striate cortex subserving foveal vision since this is an important part of the primate visual system and is a logical extension of my previous work on foveal retina. The specific objective is to understand how the perception of color and shape interact in the different layers and cells of foveal striate cortex.

Methods Employed: Our major tool is the ultrafine glass micro-pipette electrode which enables one to record electrically from outside as well as inside single neurons without significantly disturbing their function. The electrodes can be filled with conducting dye solutions (such as Procion yellow) which can be electrophoresed into a specific cell layer or in some cases inside a specific cell from which responses have been recorded. We believe this to be a powerful tool for linking structure with function in complex neural centers such as visual cortex.

An important point in my approach has been the identification of each of the three fundamental cone mechanisms of primate vision in the responses

of single cells. This is done by measuring the threshold of each cell to light stimuli from different parts of the spectrum before and after the presence of powerful chromatic adapting lights.

Major Findings: Two broad categories of cells can be identified by these methods in foveal striate cortex. One class responds strongly to certain wavelengths (colors) and not at all or only weakly to others. This group is called color sensitive. The second class responds in the same way to all wavelengths and is called color insensitive. The latter class is in general extremely sensitive to the shape and movement of the stimulus, ie. whether it is elongated, oriented, moving in one or the other direction. The former class can be subdivided into two groups one which is sensitive to the shape or movement as well as the color of a stimulus and the other which is relatively insensitive to shape and movement but sensitive to color. There is a tendency for the cells which are most sensitive to color to be the least sensitive to shape and this has suggested the hypothesis that color and form processing is ultimately carried out in separate parts of foveal striate cortex. Anatomical support for this hypothesis has been obtained. Color sensitive cells are clustered together in certain penetrations and color insensitive ones in others, indicating some vertical organization of color and shape columns. Color insensitive cells are found in relatively high concentration in layers 2 and 3 and 5 and 6. Color sensitive cells are found more often in lower layer 3 and layer 4 in foveal striate cortex indicating further anatomical differences in the lamination of the cortex for color and shape processing.

The intracellular dye injection techniques have been greatly improved during the past year so that now it has become possible to record a single cell at the same time the dye is being injected into it. It has also been discovered that small amounts (nanoamp. = 10^{-9} amperes) of hyperpolarizing current force entry of the electrode into a cell which is a great advantage. Eight cells have been marked and subsequently found. The major difficulty has been to fill fine processes sufficiently to allow unambiguous anatomical identification of the cell. What does appear to be clear is that the color insensitve, shape sensitive cells in layers 2 and 3 are pyramidal cells.

Significance to Biomedical Research and the Program of the Institute: Such studies of retinal function at the cellular level should prove valuable for understanding vision and pathophysiology of retinal disease.

Proposed Course of Project: To continue linking function with specific cells in foveal striate cortex and to understand the nature of binocular intraction on these cells. Similar types of studies are planned on the lateral geniculate nucleus and the superior colliculus.

Honors and Awards:

Invited Lecture entitled: "Color coding in Striate Cortex" delivered to the 25th International Congress of Physiological Science in Munich, July 28, 1971.

Publications:

Gouras, P.: The function of the midget cell system in primate color vision, in Symposium on Visual Processes in Vertebrates, Vision Res. Supply. 3, 397-410, 1971.

Gouras, P.: Color opponency from fovea to striate cortex, In U.S. Australian Symposium on Vision. Invest. Ophthal. (in press)

Gouras, P. and Bishop, P.O.: The neural basis of vision. Science (in press)

Serial No. NEI(I)-71 LVR 005
1. Laboratory of Vision Research
2. Section on Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: Same

Principal Investigators: Astrid Kafka, M.D. (Guest Worker)
Ralph Nelson, Ph.D. (Post-doctoral fellow)
Gunter Niemeyer, M.D. (Guest Worker)
Peter Gouras, M.D.

Other Investigators: Mary Hoff, A.B.

Cooperating Units: University of Zurich, Switzerland, for defraying costs of Dr. Niemeyer's visit to our laboratory.
University of Vienna, Austria, for defraying costs of Dr. Kafka's visit.
Patricia Grimes, A.B. and Ludwig von Sallmann, M.D. of the Lab of Neurophysiol, NINDS

Man Years:

Total:	3.2
Professional:	3.1
Other:	0.1

Project Description:

Objectives: The broad objective is to understand the mechanisms of visual processing in the retina. The current objective is to develop a system whereby it would be possible to record from within each of the different cell types in a mammalian retina and to subsequently identify them in histological sections by dye injection techniques.

Methods Employed: The principal technique involves the use of the isolated, perfused cat eye as a prototype mammalian system, a technique which has been originally developed in our laboratory. It has the great advantage of eliminating both vascular and respiratory pulsations which are a major handicap when using ultrafine pipettes to record from extremely small retinal cells.

Major Findings: A technique for recording from single cells in the inner nuclear layer of the isolated perfused cat eye has been developed. Pure rod, pure cone, and mixed rod-cone horizontal cell responses have been found. Pure cone bipolar cells have also been detected. Pure rod responses are extremely prolonged and reach a saturation level at 3-4 log units above threshold; they indicate that an isolated rod channel must exist in the external plexiform layer, an observation that ties in nicely with Kolb and Boycott's Golgi-EM studies of cat retina. Pure cone responses are much more rapid and do not show saturation unless very strong stimuli are used; these responses are found in both horizontal and bipolar cells indicating even more strongly cone independence in the external plexiform layer, and again supporting Kolb and Boycott's recent Golgi-EM data. Mixed cone-rod responses are also found, but the cone responses are in all cases much larger than those of rods. This mixing clashes with the anatomy but can be interpreted in two ways. The anatomy has missed a synaptic pathway from rods to cones or there is an artifactual reason for mixing rod and cone signals in some S-potentials. The latter possibility is developed in complete papers on this subject in preparation.

Evidence for depolarizing responses in cone S-potentials has been found. This is highly significant for it indicates antagonistic synaptic interaction in horizontal cells in the inner nuclear and external plexiform layer of mammalian retina.

A new genetic variety of retinitis pigmentosa has been discovered in Osborne-Mendel rats that has a much closer parallel to the human disease. This is being intensively studied in collaboration with Dr. L. von Sallmann and Miss P. Grimes of NINDS.

Significance to Biomedical Research and the Program of the Institute: Understanding the cellular physiology of the mammalian retina can only lead to a better understanding of abnormal states observed clinically.

Proposed Course of Project: Attempts will be made to classify horizontal and bipolar cells in cat retina by intracellular recording techniques and to continuing following up this new retinal degeneration in rats.

Honors and Awards: None

Publications:

Gouras, P., Carr, R.E. and Gunkel, R.D.: Retinitis pigmentosa in abetalipoproteinemia, effects of vitamin A. Invest. Ophthal. 10: 784-793, 1971

Gouras, P.: S-potentials. Chapter 13, In Fuortes, M.G.F. (Ed.): Handbook of Sensory Physiology. New York, N.Y., Springer-Verlag, Vo.. VII (2), 1972

Gouras, P.: Light and dark-adaptation, Chapter 16, In Fuortes, M.G.F. (Ed.): Handbook of Sensory Physiology. New York, N.Y., Springer-Verlag, Vol. VII (2), 1972

Serial No. NEI(I)-71 LVR 028(c)
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Antibody in Tears Following Intranasal Instillation of Inactivated Virus: II Use of poly I:C to Stimulate Higher Titers of Anti-vaccinia Antibody in Rabbit Tears

Previous Serial Number: Same

Principal Investigator: Harry L.S. Knopf, M.D.

Other Investigators: Morris Glassman, M.D.
Neil Blacklow, M.D.
Walter L. Cline
Vernon G. Wong, M.D.

Cooperating Units: Laboratory of Infectious Diseases/NIAID

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

The principal investigator left July 1, 1971. Project terminated.

Honors and Awards: None

Publications:

Knopf, Harry L.S., Blacklow, Neil R., Glassman, Morris I., Cline, Walter L., and Wong, Vernon G.: Antibody in tears following intranasal vaccination with inactivated virus. II Enhancement of tear antibody production by the use of polyinosinic polycytidilic acid (poly I:C). Invest. Ophthal. 10: 750-759, 1971

Serial No. NEI(I)-71 LVR 029(c)
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Antibody in Tears Following Intranasal Instillation of Inactivated Virus: III Examination of the Role of Tear Antibody in Experimental Vaccinia Conjunctivitis

Previous Serial Number: Same

Principal Investigator: Harry L.S. Knopf, M.D.

Other Investigators: Morris Glassman, M.D.
Neil Blacklow, M.D.
Walter L. Cline
Vernon G. Wong, M.D.

Cooperating Units: Laboratory of Infectious Diseases, NIAID

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

The principal investigator left July 1, 1971. Project terminated.

Honors and Awards: None

Publications:

Knopf, Harry L.S., Blacklow, Neil R., Glassman, Morris I., Cline, Walter L., and Wong, Vernon G.: Antibody in tears following intranasal vaccination with inactivated virus. III. Role of tear and serum antibody in experimental vaccinia conjunctivitis. Invest. Ophthal. 10: 760-769, 1971

Serial No. NEI(I)-71 LVR 013(c)
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Study on the Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

Previous Serial Number: Same

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure with a view toward finding more effective compounds and to possibly further the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: Studies are made on the enucleated, arterially perfused eye. Perfusate is channeled either through the ophthalmic artery in the cat to nourish the entire eye, or through one long posterior ciliary artery in the cat, monkey and rabbit to nourish the fore part of the eye which is responsible for formation of anterior chamber fluid. Drugs and other test substances are added to individual bottles of fluid which can then be introduced into the system by stopcock control. Temperature and rate of arterial flow are easily regulated. The rate of aqueous humor formation was determined by the use of I^{125} tracer (RISA).

Major Findings: Recent failure in obtaining viable preparations which would respond to drug administration, appear to have been caused by minute amounts of detergent (Sparkleen) used in washing our equipment. This problem is now solved.

Acetylcholine in concentrations as dilute as 10^{-11} g/ml in eserinized preparations produces a marked elevation of eye pressure, a decrease of blood

flow through the eye and a 3- to 4- fold increase in the rate of aqueous humor formation. The response can be inhibited by α-adrenergic blocking agents, by very dilute concentrations of atropine and by either ouabain or by acetazolamide. Presently dose-response determinations are being made on the ability of acetylcholine to increase the formation of aqueous humor. Similar determinations are being made on the antagonism of the acetylcholine response by variable dose of the aforementioned inhibitors.

Significance to Biomedical Research and the Program of the Institute: The ability of very low concentrations of acetylcholine to increase the rate of aqueous humor formation strongly suggest that a neurogenic mechanism may play a major role in the physiologie formation of aqueous humor. The ability of either ouabain or diamox to inhibit the response strongly suggests that the mechanism involved is what is generally considered as the secretory component. The fact that the response is inhibited by α-adrenergic blockade and by very low doses of atropine indicates that the site of action of the acetylcholine response is on the "E-2" sites of sympathetic ganglion-like receptors.

Proposed Course of Project: The dose-response relationships presently being performed should clarify our views concerning secretion of aqueous humor. Sympathomimetic amines will be utilized to test our presumption that the response is mediated via sympathetic ganglion-like receptors. It is hoped that we will be able to test, either here at the NIH or in collaboration with outside investigators, whether acetylcholine alters the membrane potential of the ciliary processes to further strengthen the concept of acetycholine influencing the aqueous humor secretion.

Honors and Awards: None

Publications:

Macri, Frank J.: Vasoconstriction produced in the iris-ciliary body of the cat eye by stimulation of local ganglion-like receptors. Invest. Ophthal. 10: 581-588, 1971

Serial No. NEI(I)-71 LVR 021(c)
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Clinical and Research Pupillography

Previous Serial Number: Same

Principal Investigator: David A. Newsome, M.D.

Other Investigators: None

Cooperating Units: Department of Ophthalmology, Wayne State University (Dr. Irene Loewenfeld)
NIH-NIDR (Dr. Robert Stern)

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: Clinical pupillography provides documentation, localization, and diagnosis of any lesions present in a patient's nerves of the pupillary light reflex pathway. Because the iris muscles receive either sympathetic or parasympathetic nerves, the pupil is a useful indicator for studying autonomic drugs. Specifically, the pupil has been employed to learn more about the actions of the well-known miotic, pilocarpine.

Methods Employed: Pupillary movements in response to bright light flashes were recorded simultaneously from both eyes with the electronic infra-red pupillograph and then analyzed for abnormalities of size or response. All studies were conducted on human subjects, except for certain pharmacologic experiments which were done in albino rabbits. Column chromatography and scanning spectrophotometry were also employed in the study of pilocarpine.

Major Findings: 1) in FY72 39 patients were referred from the clinical services for pupillograms. Of these, 80% had various lesions of the pupillary nervous pathway. A significant number of these lesions were not detectable by other clinical techniques.

2) In addition to its miotic action, pilocarpine diminishes the pupillary light reflex in human subjects. This effect, not previously described, has a different time course from the miosis, and, probably, a different mechanism.

3) An assay for pilocarpine using ultraviolet absorbance has been developed and used to study the effects of sera from various species and of extracts of iris tissues on pilocarpine.

Significance to Biomedical Research and the Program of the Institute: This study provides an accurate, objective method of diagnosing pupillary abnormalities, thus improving patient care. Use of the pupil as pharmacologic indicator also expands the knowledge of drugs commonly used by ophthalmologists.

Proposed Course of Project: Patients will be tested as they are referred from the clinical services.

Honors and Awards: None

Publications:

Newsome, D.A., Wong, V.G., Cameron, T.P., and Anderson, R.R.: "Steroid-induced" mydriasis and ptosis. Invest. Ophthal. 10: 424-429, 1971

Serial No. NEI(LVR)-72-112
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Neuroanatomical Connections of the Superior Colliculus in the Rhesus Monkey

Previous Serial Number: None

Principal Investigator: Marvin Snyder, Ph.D.

Other Investigators: Mortimer Mishkin, Ph.D.
Edna P. McCrane, B.S.

Cooperating Units: Laboratory of Psychology, Section on Neuropsychology, Project No. M-P-B-7.

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description:

Objectives: The anterior projections of the superior colliculus have not been previously studied in great detail. A determination of these connections is essential for an understanding of the functions of the superior colliculus.

Methods Employed: Lesions are placed stereotactically in the superior colliculus of rhesus monkeys. After survival periods ranging from seven to fourteen days the animals are sacrificed and the neural tissue prepared for use with silver impregnation techniques.

Major Findings: The superior colliculus in the rhesus monkey sends a dense fiber projection to the inferior portion of the pulvinar and also to the nucleus centralis lateralis. This finding is in accord with the results reported in more primitive mammals and suggests that the inferior pulvinar in the monkey is probably homologous to the lateral posterior nucleus in these primitive forms.

Significance to Biomedical Research and the Program of the Institute: These findings indicate the possibility of at least two pathways relaying visual information from the retina to the neocortex in addition to the classical geniculo-striate system. These proposed visual pathways would be from

a) retina----superior colliculus----inferior pulvinar-----prestriate cortex
b) retina----superior colliculus----centralis lateralis---frontal cortex
The possibility that future research may find techniques to enable the cortically blind patient to utilize the visual information in these pathways is intriguing.

Proposed Course of Project: There is a suggestion in the literature that the dorsal layers of the superior colliculus project to the inferior pulvinar (or lateral posterior nucleus) and that the more ventral layers project to the nucleus centralis lateralis. This dissociation of pathways within the colliculus will be studied in the coming year.

Honors and Awards: None

Publications: None

Serial No. NEI(LVR)-72-113
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Retrograde Degeneration in the Lateral Geniculate Body of the Rhesus Monkey after Punctate Lesions of the Striate Cortex.

Previous Serial Number: None

Principal Investigator: Marvin Snyder, Ph.D.

Other Investigators: Edna P. McCrane, B.S.

Cooperating Units: Laboratory of Psychology, Section on Neuropsychology, Project M-P-B-7.

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description:

Objectives: To study the pattern of projections of the lateral geniculate body onto the striate cortex.

Methods Employed: Small lesions, 1 mm or less in diameter, are made in the striate cortex of the experimental animals. After survivial periods ranging from six to nine weeks, the animal is sacrificed, the brain is embeded in celloidin, and every section through the lateral geniculate body is stained with thionin as is every fifth section through the lesion.

Major Findings: Contrary to popular belief, small lesions in the striate cortex of the rhesus monkey do not produce a column of degeneration extending throughout all six layers of the lateral geniculate body but may selectively involve layers 6,5, and 4, layers 6,5,4, and 3 and probably other combinations of the more ventral layers.

Significance to Biomedical Research and the Program of the Institute: These findings indicate that the presently held ideas about the pattern of projection onto the striate cortex from the lateral geniculate body must be reinvestigated. This incomplete degeneration across homologous points

in the lateral geniculate body may help to explain some of the perceptual phenomena that have been observed to take place within the area of a scotoma in human patients.

Proposed Course of Project: Lesions of varying sizes and orientations wi be made in the striate cortex in order to determine what lesion parameters ar essential to obtain degeneration throughout all six layers of the geniculate and consequently give us information about the pattern of projection of the fibers in the geniculo-striate system.

Honors amd Awards: None

Publications: None

Serial No. NEI(LVR)-72-114
1. Laboratory of Vision Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Behavioral Studies in the Rhesus Monkey Following Lesions in the Thalamus and Midbrain.

Previous Serial Number: None

Principal Investigators: Marvin Snyder, Ph.D.
Mortimer Mishkin, Ph.D.

Other Investigators: None

Cooperating Units: Laboratory of Psychology, Section on Neuropsychology, Project Number M-P-B-5.

Man Years:

Total:	4.0
Professional:	3.0
Other:	1.0

Project Description:

Objectives: To study the contribution to visual behavior and perception of subcortical structures.

Methods Employed: The monkeys are trained to solve a variety of visual problems which include pattern recognition, object discrimination, visual memory of colors, and comparing the distances between objects in space. An individual animal might receive one or more of these problems. After mastering a particular set of problems, a bilateral lesions is placed stereotactically in a predetermined subcortical structure, usually the superior colliculus or the pulvinar. After recovery from the traumatic effects of the surgery, the animal is retested on the previously learned visual discriminations and the effects of the lesion on its performance is observed.

Major Findings: Of the problems tested so far, one involving spatial perception, is particularly disrupted by subcortical lesions. Damage to the midbrain involving the superior colliculus and a small part of the central grey severely intereferes with an animal's ability to accurately estimate the distances between objects in space while leaving unimpaired other visual abilities such as pattern discrimination. Preliminary evidence indicates that the same is true for lesions in the pulvinar. Should further research confirm

this latter finding it would implicate the pathway from the superior colliculus to the inferior pulvinar to prestriate cortex as being of special importance for the perception of space.

Significance to Biomedical Research and the Program of the Institute: These findings indicate that even in the presence of a highly developed geniculo-striate system, other structures play important roles in the visual behavior of primates. These other visual systems have not received the attention that they deserve but an understanding of the function and information contribution of the various visual subsystems is essential for a development of a systems approach to visual physiology.

Proposed Course of Project: This study will continue along the same lines as more information is gathered on other visual problems and with different lesions. Of particular interest is whether the deficit we are seeing with large pulvinar lesions can be obtained when the lesions are restricted to the inferior pulvinar.

Honors and Awards: None

Publications: None

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

ANNUAL REPORT
July 1, 1971 - June 30, 1972

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

This was the Office's first full year of existence. The staff was strengthened very substantially by the addition of James Ganley, M.D, (ophthalmologist-epidemiologist), Roy Milton, Ph.D., and Rita Hiller, M.S. (statisticians).

Activities during the year included the following:

CONTINUING PROJECTS

Model Reporting Area for Blindness Statistics. 1968 data were published and 1969-70 data were edited, tabulated and prepared for publication. A decision has been made to phase out this program as its large consumption of manpower was not producing commensurate benefits.

Participation in the National Health and Nutrition Survey. This activity was transferred from the Office of the Director, NEI, during the year. Principal accomplishments were preparation of an improved protocol, initiation of a plan for replicate measurements and scheduling and training of physicians in ophthalmological residency programs around the country to conduct the exams.

Twin Studies. Several papers which raise serious questions about the generally accepted concept that the intraocular pressure response to steroids is genetically determined are either in press or will soon be ready for submission for publication. Work is continuing on the evaluation of treatment for myopia using twin pairs.

Clinical Trials. Substantial progress was made in getting the long-delayed collaborative trial of photocoagulation treatment for diabetic retinopathy to the point of starting random allocation of eyes to treatment and control status.

NEW PROJECTS

A detailed protocol and plan of procedure were developed for examining the population at Framingham, Massachusetts for glaucoma, cataract, macular degeneration, and diabetic retinopathy. A contract to conduct the study is now being negotiated. The study will provide not only prevalence data but also the opportunity to investigate whether any of these four diseases are associated with variables measured during the 20 years this population has been under surveillance by the National Heart and Lung Institute.

In cooperation with a very large diabetes clinic, detailed plans are being developed to compare diabetics of long duration who have diabetic retinopathy with diabetics of long duration who do not show these serious retinal changes. The study objective will be to try and identify factors related to the risk of developing diabetic retinopathy among diabetics of long duration.

In cooperation with an Indian ophthalmologist who has done some careful work on eye disease prevalence in the Punjab, we are making plans for a case-control study of cataract in an effort to identify factors associated with risk of this disease.

We have begun to provide statistical consultation to additional NEI clinical center activities. These include a clinical trial of soft contact lenses in the treatment of keratopathy and a review of some data collection, retrieval and tabulation procedures with preparation of plans for improvement relative to various clinical activities.

We have begun to assemble summaries of available data with regard to prevalence and incidence of ophthalmological conditions with emphasis on critical evaluation. A paper evaluating published data on U. S. glaucoma prevalence has been written and is now in press.

A study of matched pair design as related to trials of more than two treatments was undertaken to estimate the efficiency of the matched relative to the random design with respect to particular ophthalmic measurements. A paper has been written and submitted for publication.

Honors and Awards

James P. Ganley was awarded the Degree of Doctorate in Public Health by The Johns Hopkins University.

Harold A. Kahn was elected a Fellow of the American Statistical Association.

Publications (not included with individual projects):

Ganley, J.P., Smith, R.E., Thomas, D.B., Comstock, G.W., and Sartwell, P.E.: Booster effect of histoplasmin skin testing in an elderly population. Am J Epidemiol 95:104-109, 1972.

Smith, R.E., and Ganley, J.P.: Presumed ocular histoplasmosis: I. Histoplasmin skin test sensitivity in cases identified during a community survey. Arch Ophthalmol 87:245-250, 1972.

Smith, R.E., Ganley, J.P., and Knox, D.L.: Presumed ocular histoplasmosis: II. Patterns of peripheral and peripapillary scarring in persons with non-macular disease. Arch Ophthalmol 87:251-257, 1972.

Ganley, J.P., Smith, R.E., Knox, D.L., and Comstock, G.W.: Presumed ocular histoplasmosis. III. Epidemiologic characteristics of people with peripheral atrophic scars. Arch Ophthalmol (in press).

Smith, R.E., and Ganley, J.P.: Ophthalmologic survey of a community. I. Retinal abnormalities. Am J Ophthalmol (in press).

Ederer, F., Leren, P., Turpeinen, O., and Frantz, I.D.: Cancer among men on cholesterol-lowering diets. Experience from five clinical trials. Lancet, July 24, 1971, pp 203-206.

Ederer, F.: Serum cholesterol changes: Effects of diet and regression toward the mean. J Chronic Dis (in press).

Two publications of Harold Kahn's are included in the NHLI report.

Serial No. NEI (BE)-72-100

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Model Reporting Area for Blindness Statistics (MRA)

Previous Serial Number: None

Principal Investigator: Harold A. Kahn

Other Investigators: Paul A. Anderson
Helen Moorhead

Cooperating Units: None

Man Years:

Total:	5.2
Professional:	3.2
Other	2.0

Project Description:

Objectives: The purpose of the MRA study was to determine prevalence and incidence of bilateral legal blindness in the United States by etiologies and affection groups.

Methods Employed: This study was begun in 1962 by the National Institute of Neurological Diseases and Blindness in cooperation with the National Society for the Prevention of Blindness, the American Foundation for the Blind, and the U. S. Public Health Service's Chronic Disorders Division.

At the present time, blind registries from 16 states are reporting newly recorded cases of legal blindness to the MRA registry. This data is then tabulated in an annual report depicting the status of eye diseases in these participating states.

Major Findings: The data obtained from the MRA study constitutes the major source of blindness statistics in this country over the past decade.

Significance to Biomedical Research and the Program of the Institute: Blindness statistics obtained from this study have in the past provided the data for the setting of priorities in eye research and a potential instrument for evaluating how well these priorities have been met.

Proposed Course: In order to divert OBE staff to high priority epidemiological and statistical activities, OBE began to phase out its administration of the MRA in October 1971. NEI offered a contract for administering and improving the MRA to two of the voluntary organizations which are interested in blindness statistics; however, these organizations have not yet determined if they can take over the MRA activity. The final MRA statistical report which NEI will publish is in preparation and will cover Area annual data for the years 1969 and 1970. In addition to types of data previously published, the 1969-70 report will show trends over a period of several years and will include prevalence statistics for 1970 on conditions causing blindness.

Honors and Awards: None

Publications:

Anderson, P.H. (Ed.): Statistics on Blindness in the Model Reporting Area: 1968. DHEW, PHS, NIH. U. S. Government Printing Office, 1971.

Serial No. NEI (BE)-72-101

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: National Health and Nutrition Survey

Previous Serial Number: None

Principal Investigators: Arthur F. Garcia, M.D.
Harold A. Kahn

Other Investigators: James P. Ganley, M.D.
Helen Moorhead

Cooperating Units: Division of Health Examination Statistics
National Center for Health Statistics

Man Years:

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description:

Objectives: To determine the prevalence of visual disorders in a random sample of the U. S. population. Associations with nutritional defects and systemic diseases are also being studied.

Methods Employed: A random sample of 60,000 persons, from 128 geographical areas in the continental U. S., between the ages of 1 and 74, will be examined over a four-year period according to a standard protocol.

After receiving instruction in the protocol, ocular examinations are performed by house staff and research fellows from various academic institutions.

In addition to the ocular history and examination, data is gathered on medical history, dietary history, physical examination, hematologic studies, blood chemistries, and urine chemistries.

Major Findings: Data being collected.

Significance to Biomedical Research and the Program of the Institute: This is the first study to determine the prevalence of visual disorders in the U. S. population based on examination according to fixed protocol. In

addition, the study will provide a measure of the status of ocular health care, and it will provide directions for future areas of ophthalmic research.

Proposed Course: The ophthalmology exam will cease to be a part of the Survey at the completion of the first year in October 1972. This has been necessitated by an inability to obtain examining ophthalmologists. Analysis of data will be done by NEI and DHES in subsequent years.

Honors and Awards: None

Publications: None

Serial No. NEI (BE)-72-102

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Framingham Study of Senile Cataract, Senile Macular Degeneration, Chronic Simple Glaucoma, and Diabetic Retinopathy

Previous Serial Number: None

Principal Investigator: Harold A. Kahn

Other Investigators: James P. Ganley, M.D.
Thomas R. Dawber, M.D., Boston Univ. Dept. of Prev. Med.
Howard M. Leibowitz, M.D., Boston Univ. Dept. of Ophthalmology
Theodore Colton, Sc.D., Harvard Univ. Dept. of Prev. Med.

Cooperating Units: Epidemiological Research Section, NHLI

Man Years:

Total:	5.3
Professional:	1.2
Other:	4.1

Project Description:

Objectives: The aim of this investigation is to identify individuals in the Framingham Heart Study who at the present time have one of the four most common causes of adult blindness, i.e., senile cataract, senile macular degeneration, chronic simple glaucoma, and diabetic retinopathy. In addition to determining the prevalence of these diseases we hope to be able to relate past measurements to present disease status in an effort to identify risk factors.

Methods Employed: An ocular examination will be carried out according to a standard protocol by members of the Boston University Ophthalmology Department on the survivors of the original Framingham Heart Study cohort. Additional information will be obtained from data accumulated over the previous twenty years on members of this group. This data is on file with the National Heart and Lung Institute.

Significance to Biomedical Research and the Program of the Institute: The four eye diseases under consideration are the most frequent causes of adult blindness in this country today. In order to prevent the occurrence of these eye diseases, it is first necessary to identify risk factors associated with them. The study has been designed with this objective in mind.

Proposed Course: The study at the present time is in the planning stage, but it is hoped the examinations will be underway by the fall. Data will be collected over a two-year period, and data analysis will likely require an additional two years.

Honors and Awards: None

Publications: None

Serial No. NEI (BE)-72-103
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Prevalence of Chronic Simple Glaucoma in the U. S.

Previous Serial Number: None

Principal Investigator: Harold A. Kahn

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

In an effort to evaluate the commonly reported U. S. prevalence figure for chronic simple glaucoma of 2% for ages 40 and over, a critical review of the literature was made. No U. S. study was found which did not contain one or more serious flaws with respect to defined population, sampling method, sample size, disease definition, etc. Two foreign studies which met the criteria for good prevalence estimates indicated chronic simple glaucoma prevalence rates at age 40 and over of less than 1/2%. The conclusion reached was that it was reasonable to withhold judgment as to the U. S. prevalence estimates of 2% until these can be confirmed in one or more carefully conducted studies. The paper was presented at the Second Conference of the International Society of Geographical Ophthalmology in Jerusalem in August 1971. It will be published in the American Journal of Ophthalmology. This project is completed.

Honors and Awards: None

Publications:

Kahn, H.A.: The prevalence of chronic simple glaucoma in the U. S. Am J Ophthalmol (in press).

Serial No. NEI (BE)-72-104
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Efficiency of Matched Pair Design Relative to Random Design in Clinical Trials of Treatment for Eye Disease

Previous Serial Number: None

Principal Investigator: Harold A. Kahn

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Because local treatment of bilateral eye disease can easily be investigated by means of matched pair design, and since the efficiency of this design is dependent on the correlation between right and left eyes in the measure of interest (visual acuity, ocular pressure, etc.) and also on the number of treatments being studied, an investigation was begun so that future investigators would have the benefit of knowing whether matched design was or was not particularly valuable for their specific study. Methods employed were the algebraic derivation of formulae for relative efficiency and the calculation of right eye-left eye correlation coefficients for various sets of published and unpublished ophthalmic measurements. A paper on the statistical concepts involved has been prepared and submitted for publication and work continues on assembling sets of right eye-left eye data and computing correlation coefficients for them.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVE
Office of Biometry & Epidemiology

Title: Etiology of Senile Cataract

In cooperation with Dr. A. Chatterjee of Ludhiana, India, Dr. S. Franken of Groningen, Netherlands, both ophthalmologists with experience in conducting ophthalmic surveys in India, and Dr. A. Pirie of Oxford, England, a biochemist who has done extensive work on lens protein, a study of etiologic factors in senile cataract is being developed. This group met with Harold Kahn in Jerusalem in August 1971, at which time the rough outline for the study was developed. Further ideas have been exchanged in extensive correspondence since then, and Kahn will soon travel to India to develop additional details regarding the proposed research. The study outline calls for comparing 150 or more subjects who have (or have had) serious acquired cataracts without known cause with an approximately equal number of controls without any sign of cataract. Comparison will be made on many variables including diet and exposure to sunlight. The project is continuing and hopefully will develop into a PL-480 study supported by Indian rupees.

CONTRACT NARRATIVE
Office of Biometry & Epidemiology

Title: Etiology of Diabetic Retinopathy

During the year, plans were developed to conduct a case-control study aimed at discovering factors related to the development of retinopathy given the presence of diabetes of long duration. Contact has been made with the Joslin Clinic in Boston to enlist their cooperation in refining the study and also in determining the size of patient population they might be able to contribute if they were to conduct the study themselves. A small contract is presently being negotiated for various tabulations of their patient population concentrating on the number of patients they have seen in the past six months that have diabetes of 15 or more years duration without retinopathy. The project is continuing.

Section on Clinical Trials and Natural History Studies

This Office was created in May 1971 and is headed by Fred Ederer.

A large part of the year's effort of the Section was devoted to maintaining liaison with and providing statistical consultation for the Collaborative Diabetic Retinopathy Study. Contract negotiations were started toward a possible study of visual field loss prevalence with the Kaiser Research Foundation in Oakland, California. Statistical consultation was provided to several members of the Clinical Branch. A literature review is in progress of photocoagulation in diabetic retinopathy, and a statistical-methodological manuscript has been drafted for readers of the ophthalmic literature.

Fred Ederer consulted with:

Dr. Robert S. Brown, Clinical Branch, on a clinical trial of soft contact lenses.

Dr. John Marquardt, Clinical Branch, on a clinical trial of Behcet's disease and on a study of the relationship between retinitis pigmentosa and taste and smell defects.

Dr. Donald Bergsma, Clinical Branch, on a study of the ocular effects of clomid.

Fred Ederer served on the following committees:

Ad Hoc Technical Evaluation Panel, 1972 Glaucoma Contracts

Scientific review of Glaucoma Collaborative Study renewal

Executive Committee and Data Monitoring Committee, Collaborative Diabetic Retinopathy Study

Fred Ederer lectured at Howard University Medical School on "Clinical Trials."

Serial No. NEI (BE)-72-105
1. Section on Clinical Trials
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Statistical Consultation, Collaborative Diabetic Retinopathy Study

Previous Serial Number: None

Principal Investigator: Fred Ederer (this pertains to statistical consultation from the National Eye Institute only)

Other Investigators: Ophthalmologists from eight clinical centers, and personnel from the Coordinating Center, University of Maryland

Cooperating Units: Eight clinical centers, University of Maryland

Man Years:

Total:	0.33
Professional:	0.33
Other:	0.0

Project Description:

Objectives: This is a collaborative clinical trial to determine whether photocoagulation can delay the onset of blindness in diabetic retinopathy. Statistical consultation is on matters of organization, design, conduct, data collection and data analysis. The objectives are to assure adequate control of the study by the Chairman, Executive Committee, Coordinating Center, Policy Advisory Group, and National Eye Institute; improve methods of patient recruitment; develop research procedures to minimize or eliminate sources of bias; insure uniformity of terminology and definitions and standardization of methodology; monitor completeness of patient studies and follow-up; advise on data editing, monitoring and analysis.

Methods Employed: Planning for the study began late in 1968 and a detailed protocol was evolved over a 3 1/2 year period. Only patients with bilateral disease are eligible for study. One eye is randomly selected for treatment, the other is an untreated control. One of three treatments is randomly selected: argon laser, xenon arc, or combination of argon and xenon. Statistical consultation is effected through participation in the development of the protocol and operations manual, membership on the Executive Committee, Data Monitoring Committee, and site visit team.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three in that it affects a younger population. There is a real need for finding a treatment which delays the onset of blindness. Although photocoagulation is extensively used as a treatment for diabetic retinopathy, it is not known whether the patient benefits from the treatment.

Proposed Course: Patient recruitment began in April 1972. The goal is 1,884 patients to be achieved by June 1974. Each patient will be followed for five years. Expected completion date, including data processing and report writing, is 1981.

Honors and Awards: None

Publications: None

Serial No. NEI (BE)-72-106

1. Section on Clinical Trials
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Photocoagulation in Diabetic Retinopathy: Effects on Visual Acuity. A Critical Statistical Review.

Previous Serial Number: None

Principal Investigator: Fred Ederer

Other Investigators: Carl Kupfer

Cooperating Units: None

Man Years:

Total:	.20
Professional:	.15
Other:	.05

Project Description:

A large number of studies of photocoagulation in diabetic retinopathy have been reported. The design of many of these studies has not included the features generally considered essential to the unbiased assessment of efficacy, such as a control group, randomization, blind evaluation of results, and careful data analysis. Most reports are confined to the assessment of anatomical results. The purpose of this study is to critically examine the findings in light of the design and methods used, with particular emphasis on the visual acuity findings. An analysis of four studies, including a detailed re-analysis of the data in one report, has been completed. Work is continuing and will result in at least one manuscript for publication.

Serial No. NEI (BE)-72-107

1. Section on Clinical Trials
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Shall We Count Numbers of Subjects or Numbers of Eyes?

Previous Serial Number: None

Principal Investigator: Fred Ederer

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.05
Professional:	0.05
Other:	0.0

Project Description:

This is a didactic paper to alert the research ophthalmologists that it is not sufficient to report only the number of subjects studied or only the number of eyes studied, but that it is necessary to state both. Moreover, it is important to distinguish between paired and unpaired eyes, because the amount of information conveyed by paired eyes is often different from that conveyed by unpaired eyes. A manuscript has been circulated for review.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology
Section on Clinical Trials and Natural History Studies

Title: Prevalence of Visual Field Loss, Kaiser Research Foundation

Groundwork has been laid for a possible study of visual field loss in collaboration with the Kaiser Research Foundation in Oakland, California. The persons to be surveyed were aged 40-59 when they participated in the 1964-65 Kaiser Health Plan San Francisco Bay Area multiphasic screening examination, which included tonometry. A sample of some four thousand persons would be called back for perimetry. The objective is to study the relationship between field loss development and baseline intraocular pressure (IOP), drug usage, glucose tolerance, blood pressure, etc., in an attempt to identify factors prognostic of field loss.

Before deciding whether or not to proceed with the main study, a 2-stage feasibility study is planned. If the results of either stage are unsatisfactory, the main study will be abandoned. The first feasibility stage calls for a classification of IOP by age and sex to determine whether adequate numbers in various IOP groups are available for study. The second feasibility stage is to call back a probability sample of 100 persons for perimetry to evaluate the dropout and cooperation rate. Even if the main study is found to be not feasible, the data obtained in the first stage, IOP distributions by age and sex and left-right IOP correlation coefficients, will in themselves by valuable.

The Kaiser Research Foundation has submitted a contract proposal for the first stage.

ANNUAL REPORT
July 1, 1971 - June 30, 1972

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

Section on Ophthalmic Field and Developmental Research

I. Progress

During the year, the professional staff of the Section consisted of Dr. J. T. Schwartz with the part-time assistance of Mrs. Doris Collie, Medical Technician. The Section continued to operate under instructions to undertake no new investigations or professional commitments outside of the Institute. Work was carried out in the following areas: (A) Twin Studies and (B) Other Activity.

A. Twin Studies

The present Twin Register for Eye Examinations was maintained and active investigations were continued. No expansion was undertaken. Current investigations are as follows:

1. Effect of Treatment on the Progression of Myopia

Myopia or nearsightedness is the world's most common cause of defective vision. Clinical methods of controlling accommodation are sometimes employed in an attempt to retard the progression of myopia but the effect of such treatment remains unsettled.

This is a prospective study undertaken to assess the effect of a specific treatment in retarding the progression of myopia. The study population consists of 25 pairs of young monozygotic twins who are similarly myopic. One twin receives standard spectacle correction as the control; the other is given specially prescribed bifocal spectacles and topical, short-acting cycloplegic eyedrops.

During the past year, the complete study population was examined in follow-up at 6-month intervals. It is planned that the participants be maintained on the present case/control regimen for a minimum of three years. At the conclusion of the study, the progress of myopia among those receiving special treatment will be compared with the progress among those wearing standard spectacles.

2. Heritability of the effect of corticosteroids on intraocular pressure

This investigation was designed to examine a popular hypothesis that the ocular hypertensive response to topical steroids is

inherited as a simple autosomal trait. This hypothesis bears directly upon a current and widely accepted concept which ascribes a genetic etiology for chronic simple glaucoma.

Data on ocular pressure response to steroid provocative testing collected among a study population of 158 participating twins were analyzed during the past year with regard to estimates of heritability. This phase of the data analysis comprised the main focus of attention of the Section Chief during the past year and was completed as planned.

Observed estimates of heritability were low, thereby failing to support a strongly dominant role of inheritance in determining the steroid response. This observation suggests that non-genetic factors play a major role in determining variation in the ocular pressure response to topical steroids. This new finding is at variance with the widely accepted genetic hypothesis and marks the need for further investigation of the determinants of the steroid response. Appropriate manuscripts were prepared for publication and the findings were circulated widely to obtain thorough critical review.

3. Dermatoglyphics and the determination of twin zygosity

This study is directed toward establishing criteria for determining the zygosity of twins on the basis of fingerprint data. Finger and palm prints were prepared for over 1,000 members of the twin register. In collaboration with the National Institute of Child Health and Human Development (HD-CD8(c)), multiple dermatoglyphic characterisitcs are being studied in detail to determine the extent of congruency for monozygotic and dizygotic twins. Such analysis may permit a reduction in the probability of error involved in the diagnosis of zygosity by blood typing alone. This would be of value for studies of eye disease as well as other conditions.

4. Collaborative study of X_g^a blood group incompatibility in fetal loss

Findings of a recent twin study on X_g^a blood group incompatibility, undertaken in collaboration with the Children's Diagnostic and Study Branch, National Institute of Child Health and Human Development, were described in a preceding annual report. A protocol for a follow-up family study on X-chromosomal inactivation was executed during the past year in collaboration with the same coinvestigators.

5. The heritability of human salivary isoamylase

A study of the heritability of human salivary amylases was undertaken in collaboration with the Human Genetics Branch, National Institute of Dental Research. Results of studying

salivary specimens from 183 pairs of twins suggested essentially complete genetic determination for one of eight separable amylase fractions. This pattern of heritability for the salivary amylases has not heretofore been described. The findings of this investigation were published during the past year.

B. Other Activity

Collaboration, consultation and services rendered to other groups

The Head of the Section on Ophthalmic Field and Developmental Research serves as consultant to the Department of Ophthalmology, USPHS Hospital, Baltimore, Maryland; as Clinical Assistant Professor of Ophthalmology, George Washington School of Medicine, Washington, D.C.; and as consultant to the National Health Examination Survey, National Center for Health Statistics, HSMHA, PHS. He also serves on the Committee on Standardization of Tonometers, American Academy of Ophthalmology and Otolaryngology. This Section currently undertakes active collaboration with sections of the National Heart and Lung Institute, National Institute of Child Health and Human Development, and the National Institute of Dental Research.

II. Proposed Course for the Section

When activity of this Section is expanded, studies as described in the last annual report will be proposed for early consideration. These include a study of the heritability of components of refraction, a collaborative study on the treatment of myopia and a study of viral antibodies in acute iridocyclitis.

Serial No. NEI (CF) - 70 E 001
1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Twin Register for Eye Examinations (TREE)

Previous Serial Number: Same

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: To maintain a local register of twins as a resource for investigations on the heritability of ocular characteristics, case-control studies and studies of the early natural history of chronic disorders.

Methods employed: This Section has compiled a register of over 700 pairs of monozygotic and dizygotic twins for the purpose of ophthalmic investigations. All twins reside in the metropolitan Washington, D.C. area. The twin population is interviewed and given a general eye examination. Blood type, fingerprints, and ocular photographs are obtained. On the basis of information developed through the first examination, subsets of the twin population are selected to participate in specific investigations.

Major findings: A series of twins with normal visual acuity and negative history for eye disease has participated in a study of the inheritance of the hypertensive reaction to topically instilled corticosteroid (steroid) eye drops. A subset of young identical twins concordant for myopia has been assembled to study the influence of treatment on the progress of myopia. A series of 52 twinships with strabismus has been identified for study of inheritance of this disorder. A series of adult monozygotic and dizygotic twins has been studied for inheritance of AC/A ratio and other parameters of ocular motility.

The following investigations are being undertaken in collaboration with other Institutes: (1) a study of fetal loss associated with X_g^a blood type incompatibility between mother and offspring, undertaken in collaboration with the Children's Diagnostic and Study Branch, National Institute of Child Health and Human Development, (2) a study of the heritability of blood lipid characteristics being undertaken in cooperation with the Molecular Disease Branch, National Heart and Lung Institute, (3) a study of genetic factors in cardiovascular diseases being undertaken by the Field Epidemiologic Research Section of the NHLI, (4) a study of the criteria for determining the zygosity of twins on the basis of fingerprint data, being undertaken in collaboration with the Children's Diagnostic and Study Branch, NICHHD, and (5) a study of the heritability of human salivary amylases undertaken in conjunction with the Human Genetics Branch, National Institute of Dental Research.

Significance to biomedical research and the program of the Institute: Comparison of agreement among monozygotic and dizygotic twins with regard to physical characteristics is valuable as an indication of the relative roles of heredity and environment in the expression of these characteristics. This register serves as a resource to identify appropriate populations for such studies as well as investigations on therapeutic effectiveness.

Proposed course: It is proposed that this twin register continue to be maintained and expanded as a resource for direct and collaborative clinical investigation.

Honors and Awards: None

Publications:

Wolf, R.O., Taylor, L.L., Niswander, J.D., and Schwartz, J.T.: The heritability of human salivary amylases. Arch Oral Bio 16:1357-59, 1971.

Additional publications from the Twin Register for Eye Examinations appear under individual project reports.

Serial No. NEI (CF) - 70 E 002
1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Heritability of the Effect of Corticosteroids on Intraocular Pressure

Previous Serial Number: Same

Principal Investigators: Frank H. Reuling, M.D.
J. Theodore Schwartz, M.D.

Other Investigators: Manning Feinleib, M.D.
Robert Garrison, M.S.
Doris J. Collie

Cooperating Units: NAS-NRC Twin Panel
Epidemiologic Research Section, NHLI

Man Years:

Total:	1.1
Professional:	0.8
Other:	0.3

Project Description:

Objectives: To assess the role of genetic factors in determining the intraocular pressure response caused by topical application of steroid eye drops. Humoral and metabolic factors which may correlate with the steroid response are also being studied.

Methods employed: A sample of 79 pairs of monozygotic and like-sex dizygotic twins over 15 years of age were examined according to a standard protocol. Dexamethasone 0.1% eye drops were instilled three times per day for four weeks and the examination was repeated. Data were gathered on family history of various diseases, various measures of intraocular tension before, during, and after four weeks of steroids, and anatomical observations such as gonioscopy, corneal thickness, cup/disc ratio were recorded. In addition, blood chemistries including postprandial glucose and lipoprotein fractions were obtained. Physical examinations were performed by members of the Field Epidemiological Research Section of the NHLI.

The protocol for this study was approved by the NAS-NRC Follow-up Agency which granted access to those twins in their panel who reside in the Washington-Baltimore metropolitan area. Five pairs of these twins are included in the study.

Major findings: Low estimates of heritability were found which fail to support a strongly dominant role of inheritance in determining the standard parameters of steroid response. Results of the study suggest that nongenetic factors play a major role in determining variation in the ocular response to a 4-week course of topical 0.1% dexamethasone.

Significance to biomedical research and the program of the Institute: Correct assessment of the role of inheritance of the "steroid response" is of major importance insofar as this phonomenon has been described as being associated with the occurrence of chronic simple glaucoma, phenylthiourea taste testing, diabetes mellitus, thyroid function, and myopia. An important and widely held hypothesis which regards chronic simple glaucoma as being monogenically inherited was developed earlier, based on an observed familial occurrence of steroid responsiveness. On the basis of the new findings of this twin study, however, it seems evident that a theory of simple monogenic inheritance of the steroid response can be questioned. The results of the present study mark the need for further investigation of the determinants of this clinically important phenomenon.

Proposed course of project: Results of the data analyses regarding heritability of the steroid response are in press. Reports on aspects of the findings will be presented at the Seventy-Seventh Annual Meeting of the American Academy of Ophthalmology and Otolaryngology and at the Seventh Annual Joint Meeting of the U. S. Public Health Service Commissioned Officers' Association and Clinical Society. Statistical analysis of additional clinical observations as they relate to the steroid response will be undertaken during the next fiscal year.

Honors and Awards: None

Publications:

Schwartz, J.T., Reuling, F.H., Feinleib, M., Garrison, R.J., and Collie, D.J.: Twin heritability study of the effect of corticosteroids on intraocular pressure. J Med Genetics (in press).

Serial No. NEI (CF) - 70 E 004
1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effect of Treatment on the Progression of Myopia

Previous Serial Number: Same

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: To assess the effect of a specific treatment in retarding the progression of myopia.

Methods employed: This is a three-year study among a population of 25 pairs of young, monozygotic twins who are similarly myopic. One cotwin receives standard spectacle correction as the control; the other is managed using specially prescribed bifocal spectacles and topical, short-acting cycloplegic eye drops. The essential advantage in working with MZ twins in this investigation lies in the complete match on genetic constitution for the treated twin and his cotwin control. Key biologic variables of age, race, sex, period of gestation and maternal age are inherently controlled as are certain environmental factors common to the shared domicile. The study population was selected from our Twin Register for Eye Examinations.

Historical data including maternal, perinatal, growth history, family history, diet, development and past medical and ophthalmic history have been obtained. Detailed general ocular examination was undertaken. Critical measurements include refraction, corneal curvature, corneal thickness, anterior chamber depth, anterior lens curvature, posterior lens curvature, lens thickness, vitreous length and overall axial length.

Photographic and ultrasound systems were assembled for the purpose of measuring the size of intraocular compartment and the curvature of refractive surfaces of the eye. These hardware systems were pretested, base-line measurements were obtained on participating twins, and the study population was placed on appropriate treatment/control regimes. During this past year the twins were re-examined in follow-up.

Major findings: Study in progress.

Significance to biomedical research and the program of the Institute: Myopia is by far the world's most common cause of defective vision. Among environmental factors of suggested etiologic importance, one widely held theme, recurrent throughout the literature, relates the progression of myopia to prolonged use of the eyes for near tasks. Methods of treatment have been directed toward limiting accommodation and the effort of near work. Although clinical impressions and published data regarding the effect of such treatment are promising, the role of controlled accommodation on the progress of myopia remains unsettled. This study will provide a careful appraisal of the effectiveness of a clinically acceptable method of controlling accommodation.

Proposed course: The study population will be re-examined at least twice per year for a minimum of three years.

Honors and Awards: None

Publications: None

OFFICE OF INFORMATION

ANNUAL REPORT
OFFICE OF INFORMATION
NATIONAL EYE INSTITUTE
July 1, 1971 - June 30, 1972

The addition of two information specialists to the Office staff during the latter part of the year made possible the more efficient handling of press and public inquiries, management of publications, and provision of editorial and art services for the Institute. They have also enabled an expansion of activities which have a direct bearing on informing the public about vision research and the role of the National Eye Institute in fostering progress in this field.

PUBLIC INQUIRIES

Letters and telephone calls from the public continue to account for a major portion of staff effort. Letters requiring an individually written reply totalled 310; another 104 were referred to other government agencies and private organizations. Approximately 800 telephone inquiries were handled by the staff during the year, including 30 from Congressional offices. The number of Congressional and other controlled correspondence handled totalled 25.

Although the handling of written public inquiries has been facilitated by the use of the MC/ST typing system, the majority of such letters ask specific questions which cannot be answered routinely but require an individualized response. Telephone inquiries take a great deal of staff time and often interrupt other activities. Staff has been instructed to handle such calls as expeditiously as possible, but the need to be responsive and courteous to callers often results in lengthy conversations. However, the Information Officer is now handling very few of these inquiries.

PRESS RELATIONS

Six press releases were prepared during the year announcing new Council members, staff appointments of Dr. Kinoshita, Dr. Schwartz, Mr. Parish, awarding of a contract for studying congenital eye disorders (Jacobson), and announcing the 1968 MRA report. The Office assisted press representatives from CBS News-Washington, Pediatric News, The Blue Sheet, Lions Magazine, Medical Tribune, Military Medicine, The Washington Post-Los Angeles Times Syndicate, Newhouse News Service, Changing Times, and the Associated Press. The latter resulted in an AP story by science writer Frank Carey on new developments in eye research and vision substitution systems which appeared in approximately 300 newspapers around the country mentioning the National Eye Institute and quoting Dr. Kupfer.

PUBLICATIONS

The Office continued to distribute stocks of publications inherited from NINDS and Regional Medical Programs Service, in addition to the new MRA Report. The following number of publications were distributed:

Eye Research	140
Security is an Eye Patch	45,000
Refractive Anomalies of the Eye	20
MRA Report	500

In addition, 150 copies of Search for Health columns prepared by the Office for distribution through the NIH Feature Service were mailed out to individuals as fact sheets on eye disease. It is worth noting that approximately 120,000 copies of "Security is an Eye Patch" remain. This continues to be a very popular publication and demand for it continues without any attempt by us to publicize its availability. During the coming year the Office will explore the possibility of printing a similar publication, although we understand that the illustrator, Charles Schulz, and the feature syndicate which represents him have declined in the past to permit further use of the Peanuts characters in this manner.

Sixteen manuscripts prepared under contract dealing with eye diseases were received by the Office during the year. These are of varying quality and require extensive editing before publication can begin. The Office plans to issue at least three of these publications early in the coming fiscal year.

RADIO AND TELEVISION

The Office arranged a television appearance by Dr. Kupfer on the "Issues" program produced by WRC-TV, Washington, in cooperation with the National Presbyterian Center. Dr. Kupfer participated in a panel discussion titled, "Your Eyes", along with a representative of the Food and Drug Administration and a private practicing ophthalmologist. Dr. Kupfer also taped a discussion of current research on eye disorders for broadcast on the Voice of America. The Office arranged for a telephone interview with Dr. Paul Bach-y-Rita, developer of the television vision substitution system, which was distributed by the NIH Office of Information to several all-news radio stations across the country. Discussions with producers of the Marcus Welby, M.D. tv show for a program dealing with eye disease and the National Eye Institute unfortunately did not bear fruit.

MISCELLANEOUS

In addition to articles appearing in the NIH Record, resulting from NEI news releases and weekly reports, the Office prepared three stories especially for the Record. The annual Save Your Vision Week Proclamation was prepared for the White House. A message for Secretary Richardson to the Western States Optometric Congress was drafted, and background material for Dr. DuVal relating to the opening of the Oklahoma Eye Research Institute at the University of Oklahoma was prepared. Five weekly (now monthly) reports were prepared and the Office developed NEI's contributions to the NIH Almanac and DHEW Annual Report. The Office coordinated Institute submissions to the NIH Scientific Directory and Bibliography, as well as this Annual Report.

The Office of Information staff has taken an increased role in assisting other segments of the Office of the Director in preparing planning documents and budget materials, including Research Highlights submitted for the

appropriation hearings.

PROBLEMS

The doubling of the Office's staff has eased our previous personnel shortage and should permit greater activity during the coming fiscal year. However, with the addition of two information specialists the burden on our editorial clerk for final typing has increased significantly to the point where she now spends almost 100% of her time in this way. The at least part-time services of a clerk typist would be extremely helpful and would permit a more rapid outflow of materials from the Office. The distribution of publications is becoming increasingly difficult. When the Institute's new publications are available for distribution, it is likely we will need to let a contract to handle addressing and mailing. Personnel and space are adequate now for the present level of activity. However, any major expansion into large-scale media campaigns, production of films, or increased publication activities would necessitate additional professional and clerical staff.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

STATEMENT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS

A. Reorganization of Extramural Programs

I. During this fiscal year the Office of the Associate Director for Extramural and Collaborative Programs was created with NIH approval established replacing the Office of the Associate Director for Extramural Programs. This reflects the combining of contract and extramural activities in one administrative unit. Another reorganization of the Office established two branches:

1. Scientific Programs Branch: This branch has assumed responsibility for contracts as well as for grants. One health scientist administrator, in addition to other branch responsibilities, acts as coordinator for all NEI contract projects and serves as the executive secretary responsible for the scientific review of all NEI contract proposals. Qualified investigators from throughout the NEI are appointed as project officers.

2. Contracts and Grants Branch: This new branch, which replaces the former operations branch, will focus on the managerial aspects of the extramural and collaborative programs of the Institute and will encompass the processing and maintenance of contracts and grants.

II. A major reorganization of staff responsibilities took place within the Scientific Programs Branch during this fiscal year. In the past, each professional staff member was assigned grants on the basis of university location without regard to content. This has now been changed to reflect a greater awareness of program interest and to facilitate analysis and evaluation. For administrative purposes, four major program areas have been established using an anatomic classification. These are described below:

1. Retina and Retinal Diseases including chemistry of visual pigments, photochemistry, and other aspects related to the chemistry and biochemistry of the visual process at the level of the retina; retinal circulation and retinal diseases; e.g. retrolental fibroplasia, diabetic retinopathy, photocoagulation, angiography, etc.

2. Cornea, Lens, Anterior Segment and Associated Diseases and Glaucoma including lens - all aspects such as disease, biochemistry, transport; cornea - includes conjunctiva, transplants, immunology, viral infection, trachoma, transport, hydration, structure, etc; glaucoma - all aspects such as aqueous humor, anterior and posterior chamber, some studies on iris, ciliary body, autonomic innervation, intraocular pressure, etc; and combinations of areas, e.g. iris, uveitis.

3. Sensory Motor Aspects of Vision and Associated Diseases including oculomotor - neuromuscular aspects, extra ocular muscles, strabismus, nystagmus; neurophysiological and/or psychophysical studies involving

neural aspects of vision proximal to the retina; binocular fusion, development of neural pathways, etc.; some combination of motor and nerve studies; and dark and light adaptation and color vision, psychophysiology, behavior, etc.

4. Multicategorical-Multi-site Studies including bioengineering of devices for testing or treatment; development of biomaterials; evaluation of drugs and poisons on the eye as well as mechanical injury; developmental studies and congenital aspects of diseases of the visual system as well as primary genetic studies; studies involving many diseases of the eye and the visual system; ocular tumors, many anatomical sites such as lens, vitreous, and retina; studies of systemic diseases and their effects on ocular tissue, and eye care and rehabilitation.

B. Administrative Highlights

The Institute has established a new advisory body, the Scientfic Directorate, composed of senior Institute staff, to develop contract plans and priorities for the Institute as well as to make final recommendations for the award of all NEI contracts. The Associate Director for Extramural and Collaborative Programs has been appointed Chairman of this Committee.

The Institute implemented its new procedures for administering collaborative projects with the initiation of the Diabetic Retinopathy Collaborative Study. This large endeavor, which involves many institutions providing data for central analysis, requires a greater involvement of institute staff than does conventional research projects. In addition to assigning a project officer from its Extramural Program, the Institute has assigned a statistician from its Office of Biometry and Epidemiology to monitor the progress and effectiveness of this study. Acting in conjunction with Diabetic Retinopathy participants, and with the knowledge and concurrence of the National Advisory Eye Council, the Institute has accepted a recommendation to increase the number of participating clinics from ten to sixteen in the very near future. Applications have been solicited during this fiscal year for consideration at the June 1972 meeting of the NAEC.

At the March meeting of the National Advisory Eye Council, an Executive Session on training was held to discuss the reorientation of the Institute's training program from the support of clinical training to the support of research training. The current status of the Institute's training program was discussed and certain significant features were identified:

1. The continuing need to negotiate non-competing grants in order to maintain a minimum level of support;

2. the heavy commitment of training grant funds to the support of personnel, reaching 62% of total expenditures in fiscal 1971, versus 12% for stipends; and

3. the heavy concentration of salary support for those individuals who appear to be non-research oriented.

Certain elements considered essential for effective research training were identified for the Council and considered as a means of implementing training guidelines adopted in March 1970 by the Institute.

(a) There must be a high quality of on-going laboratory and/or laboratory and clinical research in the department. All trainors on the program must be active investigators, holding their own research support or capable of earning their own support. With this approach, it is possible to assure that the trainee will receive his experience in a research-oriented environment from an individual with a strong research commitment. Salary support for professional staff should be limited only to those actually serving as preceptors and trainors.

(b) There must be adequate research training facilities, including a research laboratory.

(c) There must be a well-balanced research training program sufficient in breadth of exposure and period of training to produce ophthalmologists highly qualified in research capabilities. Training experiences both in laboratory and in clinical research are essential. Hence, training programs may well be at a two to three year duration, but independent of residency training.

C. Funding of Research and Training Grants

(Amounts in thousands)

	Noncompeting		Competing Renewals		New and Supplemental		Totals	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Research	289	16,505	55	3,567	82	3,327	422	23,399
Fellowships	15	338	7	68	100	1,270	122	1,676
Graduate Training Grant	31	2,229	11	547	4	222	46	2,998

For the third year, the Institute implemented a plan for coping with the deficit in funds available for training grants. The plan called for (a) reduced funding of noncompeting grants by an average of 18% and, (b) reduced funding of competing renewal grants by order of priority score to the extent made possible by the remaining funds. Following this plan,

the Institute was able to award 10 renewal applications for on-going training programs and five new applications. Only one approved (new) training grant application went unfunded.

D. Staff Changes

Within the past year, Dr. Samuel Schwartz was appointed Chief of the Scientific Programs Branch and Deputy Associate Director for Extramural and Collaborative Programs. He replaces Dr. Samuel Price who left to take a position with the National Cancer Institute. Mr. George Parish was appointed Chief of the Contracts and Grants Branch. Mrs. Betty Connolly has assumed committee management responsibilities for the NEI replacing Mrs. Gerry Benson who retired. Dr. George Riley, Health Scientist Administrator in the Scientific Programs Branch, was detailed to the National Heart and Lung Institute for an extended period to assist with the sickle cell anemia grants program.

ANNUAL REPORT
JULY 1, 1971 THROUGH JUNE 30, 1972
EXTRAMURAL PROGRAMS

Program Report

By use of grant and award mechanisms the NEI Extramural Program area supports investigations into the complex disorders which manifest themselves in terms of reduced vision and/or total loss of sight. The NEI has been developing a broad scope of clinical and laboratory research projects which utilize the full range and broad base of scientific disciplines.

For the purposes of this report there has been a selection of areas of investigations which represent progress in research sponsored by NEI. This aspect of the report is organized in the following manner:

I. Diseases of Anterior Segment

II. Diseases of Posterior Segment

III. Disorders of Nervous Elements and Neural Pathways

IV. Eye Movements and Their Disorders

V. Devices for the Blind

VI. Collaborative Studies

VII. Contracts and Developmental Research

I. Diseases of the Anterior Segment

The eye-globe lies within a bony cavity known as the orbit. The contents of the eye are covered by a tough connective tissue which serves to protect the visual apparatus. The anterior segment of the eye consists primarily of the optical media through which light will pass and be focused on the retina. The front part of the outer coat has a transparent area which is called the cornea. If the path of light energy were followed through the anterior segment, it would pass through the transparent cornea, into a fluid known as the aqueous humor, into the ocular lens and on to the posterior segment. The aqueous humor is produced by mechanisms of active secretion and ultrafiltration from the ciliary body which lies behind the iris. This fluid contributes to the normal physiology of the lens and cornea which are avascular structures. The aqueous humor secretion and outflow achieve a balance which contributes to the normal pressure relationships within the eye-globe.

Disorders of Cornea - The cornea is composed of a stroma, its overlying epithelium and its underlying endothelium. The latter tissue consists of one layer of cells bathed by the aqueous humor. Normal transparency of the cornea is dependent upon the precise regulation of its water and salt contents. The cornea is maintained in a deturgescent state by the action of a "metabolic pump" associated with the endothelial cells. The NEI is sponsoring studies conducted by Dr. David Maurice, Stanford University, on the metabolic requirements of the endothelial "pump." Ingenious methods have been developed for investigation of fluid transport across the cornea. It has been demonstrated that such transport can be maintained in a simple balanced salt solution medium. This information is useful in understanding the causes of corneal edema and opacification. These studies offer a rationale for treatment of pathologically cloudy corneas. This information also has practical implications for the assessment of corneal donor eyes. Studies with rabbit corneas preserved at low temperatures in suitable fluids, as determined by the above experiments, showed better than 90 percent successful transplantation after 10 days of preservation. When the "metabolic pump" mechanism fails, edema results, and there is a decrease in corneal transparency and a loss of vision.

In order to draw water from the cornea to offset the malfunctioning "pump," it is necessary to apply hypertonic saline to the corneal epithelial surface, so that it is continually bathed. The advent of the hydrophilic contact lens has provided a means of achieving this goal. The lens can be worn for prolonged periods, and thus maintain a hyperosmotic medium upon the surface of the cornea. The combination of a hydrophilic contact lens and topically applied hypertonic agents can result in significant visual improvement in certain cases of corneal edema, as demonstrated by Dr. Howard Leibowitz, Boston University, and Dr. Herbert Kaufman, University of Florida.

Investigations continue into the problem of the usefulness of hydrophilic contact lenses as a therapeutic adjunct in the management of several types of corneal disorders. In selected patients with early bullous (characterized by blisters), keratopathy and minimal stromal scarring, NEI-supported studies show that vision could be improved if the hydrophilic lens were worn continuously and 5 percent saline drops were concomitantly administered every two hours during the day. Further studies demonstrated that cases of traumatic penetrating corneal lacerations could be treated primarily with a hydrophilic contact lens, and thus avoid the need for surgical closure. The hydrophilic lens can successfully tamponade the leaking corneal wound and produce rapid restoration of the anterior chamber. Wound healing can then proceed and excellent visual results achieved.

Progress in the field of corneal preservation was the subject of a recent symposium held at the University of Florida. Several NEI supported groups have shown that morphological changes after cryopreservation are small. Those changes which are seen, are generally reversed very rapidly once the preserved corneas are grafted into the animal eye. These studies suggest that effective cryopreservation of human corneas has a sound laboratory as well as clinical basis. The parameters necessary for successful corneal cryopreservation, both in terms of the solutions necessary and in terms

of the required freezing curves, have been defined. In order to make the technique more practical, a rather simple inexpensive freezer has been built which programs the freezing curves and is now in use by a number of eye banks throughout the country. The thawing procedure has also been simplified and defined. There are ten institutions using this cryopreservation technique on a regular basis, and 25 other institutions are preparing to use the technique. The basic biology of preserved tissue, as studied by enzyme histochemistry, electron microscopy, and persistance of DNA labelled cells after keratoplasty all confirm that it is a functioning tissue.

Evidence from the work of transplantation biology has established that sensitized lymphocytes are involved in graft rejection. Studies of Dr. Frank M. Polack, University of Florida, indicate that an analogous situation occurs with corneal graft rejection. He is trying to determine how lymphocytes damage donor cells by extensively studying the earliest detectable morphologic changes in rejected corneal grafts by scanning electron microscopy. Clarifying the cell changes and the possibility of reversal of lesions or regeneration will help in planning appropriate medical treatment, and a great deal will be learned about the earliest cellular alterations induced by lymphocytes sensitized against donor tissue. Studies of unsuccessful grafts obtained from clinical material and from experiments simulating these conditions will help in identifying other causes of graft disease which are amenable to correction.

Hydrophilic soft contact lenses made from different hydrogels of hydroxyethylmethacrylate have been the subject of many recent investigations by Dr. Miguel Refojo, the Retina Foundation. Dr. Claes H. Dohlman, Massachusetts Eye and Ear Infirmary, is conducting tests to evaluate the utility of these lenses for both therapeutic and cosmetic use. Different manufacturers have varied the amount of polymer crosslinking, the polymer curing procedures, and techniques of fabrication of the finished contact lens. The resulting lenses have separate and distinct physical properties such as mechanical strength, thermal degradation, absorption and elution of aqueous solution, and degree of hydration.

An earlier study indicated that the hydrogel lenses fabricated in Czechoslovakia were as impermeable to oxygen as the methylmethacrylate contact lenses. The state of the art of hydrophilic contact lens fabrication has changed so rapidly, however, that the various lenses available today are quite different from those examined previously. The water and oxygen permeability of some of the hydroxyethylmethacrylate hydrogel materials have recently been studied by NEI grantees; however, the permeability characteristics of the currently available hydrophilic contact lenses remain to be investigated.

Drs. Dennis R. Morrison and Henry F. Edelhauser, Medical College of Wisconsin, have investigated the water and oxygen permeability of some of these hydrogel contact lenses. The oxygen diffusion characteristics of the three hydrogel lenses indicate that oxygen deprivation of the corneas should not arise from prolonged wearing and that the

oxygen starvation syndrome observed with the methylmethacrylate lenses should not occur with the use of these hydrophilic lenses.

Disorders of Lens

The ocular lens is composed primarily of protein which represents approximately 90 percent of the dry weight of the tissue. Of particular interest is the structural protein known as alphacrystallin. Studies on this protein by Dr. Abraham Spector, Columbia University, have indicated that it is composed of different sized populations of macromolecules, and investigation of the relative amounts of these populations in bovine lenses has shown that with increasing age the proportion of the higher molecular weight species increases. As the lens ages its transparency decreases and with sufficient aging an opacity will develop. It is possible that the gradual increase in the aggregate size of the alpha crystallin is the cause of the opacity associated with aging.

Light scattering studies by Dr. George Benedek, Massachusetts Institute of Technology, suggest that such macromolecules are capable of scattering light in a manner sufficient to produce cataract if they are present in sufficient concentration with a sufficiently different index of refraction from the average index of the region surrounding the aggregates.

The role of proteolytic enzymes in human lens tissue and their possible relationship to cataract formation is fragmentary but under study by NEI-supported investigators. The role of proteolysis in cataractogenesis has long been suspected but never proven nor adequately studied. A start has been made by Dr. Arnold A. Swanson, Medical University of South Carolina, in isolating and characterizing proteases from human cataractous lens and in determining the distribution of enzymes within the lens. The fact that this investigation has been carried out on human rather than on animal lenses adds to the significance of the work since there appears to be a marked species difference in these proteolytic enzymes.

Recent evidence obtained by Drs. J. A. Jedziniak and J. H. Kinoshita, strongly suggests that the enzyme aldose reductase plays a primary role in the initiation of cataractogenosis in experimental galactosemia and diabetes. Attempts have been made to seek specific inhibitors that can control the activity of the enzyme so that the cataractous process can be prevented or at least delayed. Isolation and purification of the enzyme has been undertaken, and the highly purified enzyme has been shown to be a dimer of two smaller units, each with a molecular weight of about 20,000. It is maintained in the active dimer form by the presence of sulfhydryl compounds and in their absence, the enzyme becomes inactive and splits into the smaller units. The action of inhibitors, such as tetramethylene glutaric acid, is to convert the active form into the inactive form by dissociating the enzyme into the two component subunits.

Drs. Shuzo and Kinoshita have obtained evidence regarding a recessively transmitted cataractous trait in mice which suggests that an apparent deficiency of

$Na^+ - K^+$ activated ATPase may be involved in the initiation of this type of cataract. The abnormality, demonstrable in 13 day old mice, leads to inefficiency of the cation pump mechanism. At 13 days the lens was clear and the electrolyte levels were normal. However, by the 20th day, it became apparent that the defective lens was no longer able to extrude sodium efficiently. This sudden increase in electrolytes drew water into the lens, an osmotic change occurred, and a nuclear opacity appeared. Such experiments will be useful in elucidating the etiology of cataracts in humans.

A mechanism whereby the near ultraviolet portion of sunlight can alter the properties of ocular proteins after penetrating the cornea is suggested by Dr. Seymour Zigman, University of Rochester. Such light has been found to photooxidize aromatic amino acids and other aromatic substances to form colored compounds which combine with lens and aqueous humor proteins. Irradiation of tryptophan in neutral aqueous buffers induced the formation of a yellow-brown oxidation product which has twice the molecular weight of tryptophan, no free a-amino groups and an altered absorption and fluorescence spectra. This product combined firmly with amino and sulfhydryl groups of lens crystallins and many other proteins, coloring them, and appreciably altering their light absorption and fluorescence properties, their solubilities, and their electrophoretic mobilities. Human lenses incubated in physiologic media containing tryptophan, and irradiated with near ultraviolet (UV) light as described above, became more intensely yellow-brown and absorbed blue light more efficiently. Uncolored rabbit lenses, irradiated for 72 hours in unaltered bovine aqueous humor also became yellow-brown as did the aqueous.

The thesis is that aromatic compounds are photo-oxidized in the aqueous humor and lens by the near UV components of sunlight entering the eye. These substances combine with lens proteins and contribute to an increased browning with aging, and may also result in brunescent cataracts. Other chemical changes in lens proteins, such as diminished solubility, may interfere with lens transparency. Alteration in the chemistry of aqueous humor proteins may result in the blockage of aqueous drainage channels, allergic manifestations, and altered eye tissue membrane permeabilities.

Practical application to eye health is suggested. Crystalline lens absorption protects the eye from harmful near UV energy, and aphakic persons should have near UV absorbing spectacles instead of ordinary optical glass. Xenon arc photocoagulators emit near UV energy potentially harmful to the aqueous humor and lens, suggesting a need for filtration of these wavelengths.

Disorders of Aqueous Humor Secretion

Primary open angle glaucoma is a chronic, slowly progressive, bilateral disease, characterized by elevations of intraocular pressure sufficient to produce damage to the optic nerve. It is insidious in onset and progresses imperceptibly without symptoms until characteristic field loss occurs. The disease may proceed to blindness without pain or

other symptoms. In almost all cases the elevated intraocular pressure is related to obstruction to the outflow of aqueous humor, and in many cases blindness can be prevented if treatment is instituted early. The objective of therapy is to facilitate the excretion of aqueous through existing outflow channels and in some cases to inhibit the secretion of oqueous humor by the ciliary processes using systemically and topically administered drugs. When medical management is no longer sufficient to control the intraocular pressure, operative treatment is necessary.

Dr. Morton Grant, Massachusetts Eye and Ear Infirmary, and his collaborators have been investigating the anatomy and function of the aqueous humor outflow system, particularly its physical characteristics and the manner in which microsurgical procedures may regulate the intraocular pressure. They have devised ingenious microsurgical techniques which are particularly attractive because they are concerned with the basic mechanism responsible for the glaucoma. Clinical evidence suggests that much of the resistance to outflow in the eye with open angle glaucoma is caused by decreased permeability of the inner wall of Schlemm's canal and the adjacent trabecular meshwork or perhaps by compression of the inner wall against the outer wall of the canal.

Dr. Bernard Becker, Washington University, is investigating the hereditary nature of glaucoma, modified by factors such as age, sex, endocrine status, optic nerve cupping and blood supply. He emphasizes that glaucoma should be suspected not only when elevated pressure or decreased outflow facility is found, but also when there is a large cup at the optic nerve head, a family history of glaucoma and when diabetes or decreased thyroid function has been diagnosed. Pressure sensitive field defects are reversible in the early stages. The relationships between the endocrine and ocular parameters and primary open angle glaucoma can be used clinically to increase suspicion of the diagnosis especially when multiple factors occur in the same person. Dr. Becker points out that the oppreciation of such modifying factors superimposed upon the genetic constitution of the patient may lead to a greater understanding of the glaucomatous process. They may even provide better methods for avoiding or delaying the development of glaucoma and the devastating damage it produces, in spite of the genetic background.

Since the compound diphenylhydantoin (DPH) has been shown to offer protection from the effects of anoxia or cardiac and nervous tissue, it was reasoned that if ischemia is a significant factor in damage of the optic nerve in glaucoma, some protection might be obtained by administering this drug. In a pilot study by Dr. Bernard Becker, Washington University, 21 patients with glaucomatous field loss were treated with DPH for up to five months. Nine patients showed reversal or improvement in the visual fields, four seemed to be protected against further field loss despite markedly elevated intraocular pressures, and eight showed no change in their visual field. Exploratory clinical tests support this idea and NEI is encouraging more investigations along this line.

Ocular surgical sympathetic denervation was utilized near the turn of the century in an attempt to control intraocular pressure in glaucomatous eyes. However, the procedure was soon abandoned because of the technical difficulties involved in surgical

sympathectomy as well as the lack of reliable results in controlling glaucoma. Recently there has been a revival of interest in the ocular effects of sympathetic denervation, and in the mechanism of action of sympathetic drugs on aqueous humor secretion and its outflow. 6-hydroxydopamine (6-HD) has the unique effect of producing a reversible degeneration of sympathetic nerve terminals. An investigation of its effects on intraocular pressure, facility of outflow and episcleral venous pressure of owl monkeys and New Zealand rabbits is being studied by Dr. Monte Holland, Tulane University. It is suggested that chemical sympathectomy with 6-hydroxydopamine reproduces many of the phenomena of surgical sympathectomy. It is a useful drug for experimental ophthalmology and may prove useful clinically. The procedure may be modified to explore its use in the treatment of volunteer human patients who have either been blinded by glaucoma or who have failed to respond adequately to medical and/or surgical therapy. The preliminary results of this investigation suggests that chemical sympathectomy combined with topical epinephrine therapy was successful in controlling the intraocular pressure in 11 or 12 severe cases of uncontrolled glaucoma. Although the clinical exploration of the use of 6-HD has only just begun, it appears to be a promising new approach to glaucoma therapy.

II. Diseases of the Posterior Segment

The lens of the eye focuses light on a distant area of the retina which is located temporal to the optic disc. This area is called the fovea centralis and contributes to the visual systems greatest visual acuity and color vision by virtue of the high concentration of cone (daylight) photoreceptors. Because the visual system focuses light energy in the macular region and because it is involved primarily in photopic (daylight) vision, minute lesions which might not cause visual discomfort in peripheral areas of the retina, do cause serious loss of central visual acuity and color discrimination when located in the macular region. This region of the retina is involved in a variety of retinal degenerative conditions. In addition, the macula is subject to all the pathological alterations which may develop in other areas of the retina.

Diseases of Retina

The abnormalities associated with the retina are of special concern because of the debilitating consequences of the loss of central visual acuity and depressed color vision. The NEI is supporting research in retinal diseases which have a basis in abnormal pigmentation and photoreceptor degeneration, aging and edematous degeneration and vascular proliferation and disturbances. With the exception of clinical problems due to trauma, the major diseases of retina appear to have an hereditary basis.

Dr. Abraham Kornzweig, Jewish Home for the Aged, New York, has demonstrated that macular degeneration is occasionally found in infancy, adolescence and the remainder of life with the incidence increasing to approximately 24% in populations between 65 and 80 years of age and approximately 38% in those over 80 years of age. Dr. Alex Krill, University of Chicago, has been studying inherited diseases of the eye

and has demonstrated that this progressive type of macular degeneration appears to have a familial basis, and therefore, it is a retinal defect which is genetically transmitted. Usually the familial type of macular degeneration is bilateral, progressive and is referred to as a "primary type." Dr. Edward Norton, University of Miami, has shown that this type of macular degeneration does not lend itself to known therapy. In the late stages of the disease it is usually necessary to use low-vision aids which will allow the subject to continue activities which do not require sharp vision. Further, another type of macular degeneration may develop as a result of vascular or inflammatory diseases. It is usually unilateral and may not be progressive after the primary cause has been removed and is referred to as a "secondary type."

In general, the genetic characteristics, age of onset, cause of onset, natural history, effects upon vision and therapeutic measures are poorly understood. The NEI is supporting research in the macular degeneration disease problem. However, until more information is obtained, the clinical ophthalmologists can do little more than observe the development of retinal degenerations and limit therapy to symptomatic measures.

Macular degeneration may occur in all age groups, in families, and may be secondary to other systemic and vascular disorders. NEI-supported investigators are attempting to classify the degeneration by age of onset, inheritance, by anatomical localization within the macula, by appearance of the lesion and by visual functions. Since a limited amount of post-mortem material and since no biopsy specimens have been available, it has been necessary for the ophthalmologist to deal with clinical impressions. The clinical material has varied widely in description and classification systems have been unsatisfactory. There are a few families which have been studied by Dr. Harris Ripps, New York University Medical Center, because of the presence of primary macular degeneration which manifests itself by color blindness at an early age. Such conditions can be distinguished from congenital cone abnormality by the development of pigmentation in the macula. Most color vision defects as well as some retinal disorders are thought to be sex-linked hereditary disorders; however, the sex-linked aspects are open to question. Studies of retinal disorders involve the use of psychophysical and genetic measurements for their detection and classification. In secondary macular degeneration more information is needed with regard to the underlying causes. When the causative diseases are diabetes and hypertension, the condition of the macula may be improved through study and treatment of the primary disease. Dr. Howard Leibowitz and Dr. Ronald Laing, Boston University, find that this problem may be approached through better comprehension of retinal circulation and metabolism. In some cases of macular degeneration there is a loss of fluids from the adjoining blood vessels and the accumulation of such fluids in the macular region, with a resulting edema.

Humans who are suffering from hereditary macular degeneration, senile macular degeneration, central serous retinopathy as well as other disorders of the macular region are being educated at NEI-supported outpatient clinical centers with the importance of entering controlled studies at the first sign of change in their central visual acuity or color vision. Dr. Abraham Kornzweiz finds that with the cooperation of patients seen at

specialized clinics and in homes for the aged, studies can be conducted in order to determine the natural course of macular degeneration and in order to determine the possibility of predicting the loss of central visual acuity. Dr. Edward Maumenee, Johns Hopkins University, finds that in all cases of retinal degeneration, studies of diagnostic procedures require large stable populations at clinics which use fundus photography in conjunction with fluorescein angiographyas critical tools in diagnosis, observation and confirmation of macular disorders; such studies are also being conducted, with NEI support, by Dr. Joseph Wadsworth, Duke University; Dr. Thomas Duane, Jefferson University; and Drs. Albert Potts and Frank Newell, University of Chicago. NEI-supported studies show that the use of intravenous fluorescein for retinal angiography provides the best means of observing the various disorders of the retina. Dr. Peter Evans, Georgetown University, is pursuing research into the modification of existing techniques and instruments in order to increase the resolution and detection of the smallest retinal blood vessels. The development of cineangiography is being supported as a means of evaluating the vascular physiology, since vascular leaks may be missed without observations of intravenous flow. In addition to the analysis of the patient's condition by use of fluorescein angiography and fundus photography for the visualization of leaky retinal blood vessels, other physiological studies include electroretinography as an index of the state of degeneration of the photoreceptors and are being conducted by Drs. Ronald Carr and Harris Ripps, New York University Medical Center.

The photoreceptors and nervous elements in the region of the macula are active cells and sensitive to the need for a normal blood supply and proximity of other cells. Since the reduction in central visual acuity is related to the loss of cone function, it is critical to prevent the damage to cones which cannot be restored when there is a dimunition of blood supply. In senile macular degeneration there is no current treatment which can be offered to counteract the alterations in blood supply. Although central visual acuity is significantly reduced, the patient may retain peripheral vision and can be trained so that he is not helpless. In addition, the patient may be fitted with telescopic lenses, magnifying lenses and other optical aids which are available, although under further refinement, through NEI-supported projects. In some secondary macular degenerations, choroidal hemorrhaging may cause a macular edema which gives rise to a distortion of the macular region. Dr. Christian Zweng, Stanford University, has the clinical impression that some cases which involve retinal vascular diseases may be treated with the argon laser. Further investigations are being supported in order to determine whether argon laser photocoagulation of blood vessels influences the progress of retinal degeneration involving hemorrhaging and/or leaky blood vessels. The laser can be used to obtain small retinal spots on the retina, and therefore in treatment of vessels near the fovea centralis of the macula, damage from the laser to surrounding receptors can be kept to a minimum as discrete areas are photocoagulated. The response to treatment has not been dramatic or totally convincing and is under further study through NEI support. When macular degeneration is clearly associated with vascular and metabolic disorders as the primary cause, drugs and vitamins have been explored in an attempt to treat the systemic disorder. However, some drugs which affect the cardiovascular system have no effect on the choroidal circulation. Until pharmaco-

logical agents which will act at the level of choroidal and retinal vasculature are developed, diseases of retinal degeneration associated with vascular degeneration may be without an effective treatment.

In humans who are suffering from macular degeneration, clinical specimens are being obtained for study and can be correlated with the natural history of the disease, family history, descriptions of the macular lesions, treatments attempted and classification systems. With NEI support, some eyes have been obtained after death and have been studied by routine histological techniques of staining and isolation of blood vessel networks in order to learn more about the lesions of the macular region. These studies have been inconclusive. Metabolic abnormalities associated with macular degeneration may offer some insight into disease mechanisms. An NEI-supported study has shown an amino-aciduria correlation in a few families with histories of hereditary macular degeneration. The use of high vitamin diets to counteract insufficiencies of vitamin A and B complex have been explored, without effect on the disease. Until a satisfactory animal model is developed for study in the laboratory, research must depend upon observations of a descriptive nature and must be conducted on humans on an out-patient basis.

Another among the diseases of the eye which show a familial tendency is a form of retinal degeneration which progressively worsens throughout life and results in total blindness by middle life in a small percentage of the victims. This retinal abnormality is referred to as retinitis pigmentosa. The early symptoms of this disease appear in the first decade of life in the form of night blindness. A ring of depressed vision (scotoma) may develop which then spreads peripherally and centrally throughout life until at the age of 50-60 years only a very narrow central field of vision (gun-barrel vision) will remain. This condition permits reading vision but no peripheral vision, so that movement in unfamiliar surrounding is not readily accomplished.

Families with this disease have presented themselves for study, and therefore, Dr. Alex Krill, University of Chicago, has published some pedigrees. There are families showing that the disease is inherited in a number of genetically determined fashions, viz. as an autosomal recessive, as an autosomal dominant and as a sex-linked characteristic. NEI-supported studies have shown that retinitis pigmentosa usually occurs as a single entity with bilateral loss of vision. However, Dr. David Klein, Geneva, Switzerland, has shown that the retinal disease may be part of the complex syndromes of obesity, mental retardation, hypogenitalism, polydactylism and can also be accompanied by high myopia, cataract and glaucoma.

There have been families which show the condition of sector retinitis pigmentosa. Drs. Krill and Klein find that these families also show the variety of autosomal and sex-linked inheritance. However, in the sector disease, the visual field loss does not spread uniformly across the retina in a ring form but field loss is found in patches of the retina. Nevertheless, vision loss can be detected in the "normal" areas of the retina. It becomes a matter of degree and distribution of retinal degeneration within a given diseased eye. Sector retinitis pigmentosa has been shown to be less progressive and can

be distinguished from widespread retinitis pigmentosa which is progressive, on the basis of ophthalmic and clinic tests. Reduced scotopic (rod) vision and progressive loss of peripheral fields and blurred vision are the early symptoms which the patient is aware of. Examination of the fundus at advanced symptomatic stages shows narrowing of blood vessels, a yellowing of the optic disk and deposition of pigment with irregular processes. The pigmentation of retinitis pigmentosa is distinguished from the secondary pigmentary degeneration of other diseases by pigment distribution and location, quantity of pigmentation and scotoma patterns. Ophthalmic examination of the fundus is practical in the late stages of retinitis pigmentosa, however, early detection requires more sophisticated analyses. Dr. Eliot Berson, Harvard University, has employed electrophysiological methods in the study of carriers, asymptomatic and minimally symptomatic individuals and siblings. In an effort to understand the early stages of retinitis pigmentosa, NEI is supporting investigations which use electroretinogram (ERG) as a means of differentiating retinal abnormalities. Children in families with a history of retinitis pigmentosa have been studied by Dr. Berson and show that in early stages of the disease, the ERG can be used to differentiate retinal abnormalities. Reduction in ERG a-wave amplitude indicates the photoreceptors are undergoing physiological changes which probably involve the outer segments of the photoreceptors. The ERG defects appear to exist in every genetic type of retinitis pigmentosa. The ERG in early stages of autosomal recessive retinitis pigmentosa is characterized by reduction in amplitude and a delay in implicit time of both cone and rod system responses. The ERG wave forms appear to be different in autosomal recessive and autosomal dominant retinitis pigmentosa. In patients with no previous known family history of retinitis pigmentosa, a history of recessive inheritance is suspected, and the ERG wave form defects are usually the same as those from families where recessive inheritance is established. Sector retinitis pigmentosa is minimally progressive and distinguished from the widespread degeneration by temporal aspects of ERG. In sector retinitis pigmentosa, ERG implicit times are normal while in widespread retinitis pigmentosa, ERG implicit times are delayed even when ERG amplitudes are reduced in both sector and widespread retinitis pigmentosa.

Continuous exposure to artificial light from incandescent and fluorescent lamps can produce damage to the retina, and vitamin A deficiency can also lead to retinal damage. The relationships between light, vitamin A, and retinal function in rats can be interpreted in terms of human retinal dystrophies. Rats kept in darkness or light, rats exposed to alternate periods of light and dark similar to the variations in day and night, and the effect of the presence or absence of vitamin A in the diet during these periods need to be investigated further as a possible therapeutic model. The results of these experiments indicate that the normal cyclic variation of light is essential to the health of the retina. In the absence of alternate periods of light and dark, the retina of normal rats can be abnormally sensitized to ordinary levels of light and irreversibly damaged. This information is critical to speculative treatments of retinitis pigmentosa and is being studied by Dr. Werner Noell, University of Buffalo.

The very early events which relate to retinal degeneration may be of a biochemical nature and precede the anatomical and electrophysiological events. NEI-supported

studies of temporal factors and light effects on retinal enzyme development indicate that causative mechanisms may occur in the first decade of retinitis pigmentosa, in humans. Adaptation of the retina to light and dark can be observed by recording signals from specific cells not directly involved with the primary absorption of light. Dr. John Dowling, Harvard University, and his colleagues report that retinal cells of a specific type or S-units give electrical signals similar to those seen in a specific portion of the complex electroretinogram. Adaptation to light occurs before electrical signals are emitted from these S-units. These results are part of a fund of knowledge needed to improve understanding and interpretation of the clinical electroretinogram, which may prove to be one of the critical diagnostic tools in the early assessment of retinal degeneration.

Although the involvement of other syndromes and complexity of hereditary patterns may complicate diagnostic procedures, it is urgent that current studies of retinitis pigmentosa be continued in order to diagnose the condition when children are minimally symptomatic. At the asymptomatic stage, retinal function is relatively normal and therapeutic methods may be explored.

Drs. Ronald Carr and Harris Ripps, New York University Medical Center, have been observing patients classified as unilaterial retinitis pigmentosa. Its onset is more sudden and late in life. This condition is a variant of the more common form of bilateral retinitis pigmentosa and may have its origin in a vascular disorder which may be an acquired or genetic characteristic. Dr. Harris Ripps has reported that the state of the visual processes may in part be determined and early diagnoses conducted by use of fundus reflectometry. This technique is being supported and studied by Drs. William Rushton and Howard Baker, Florida State University, and provides information about the concentration and kinetics of visual pigments. This type of information can be related to the physiological status of the photoreceptors in suspected cases of retinal degeneration. Complete diagnostic procedures will await sufficient knowledge to allow meaningful correlations of retinal electrical, photochemical and anatomical events in normal and degenerative retinas. Dr. Eliot Berson reports that large numbers of families are entering controlled studies in order to establish a base for the differential diagnoses of retinitis pigmentosa. In this manner genetic counseling may be provided. Early diagnosis will allow exploration of therapeutic regimes which may relate to the effects of light, nutrition and drugs with unique affinities and effects on the retina.

The sequence of events which occur as the retina is formed, and as it renews itself once formed, need to be better understood. Investigations which trace the poth of biologically active molecules in the retina from synthesis to degradation have as a major objective to clarify the metabolic activity and function of the specialized cells in the retina. NEI is actively supporting studies in this area of research. Drs. Dean Bok, Michael Hall and Richard Young, U.C.L.A., have been studying the relationship between the layer of cells next to the retina (pigment epithelium) and the light receptor cells, the rods and cones. These investigations have a direct bearing on retinal diseases. The light-sensitive outer segment of the rod cell is composed of hundreds of

discs. NEI-supported investigators have shown that each retinal rod produces the discs which form its outer segment. The continued production of new discs displaces the previously formed ones which are pushed gradually towards the pigment epithelium where they are phagocytized. Thus, the entire rod outer segment is renewed. Older discs are shed and consumed by the pigment epithelial cells. This renewal process does not occur in the cone cells. These NEI-supported findings provide new and important information about the metabolic changes that occur in the normal retina during development and have an important bearing on the causes of hereditary retinal diseases in man. The hereditary retinal disorder which occurs in a special strain of rats has been regarded as a model for retinal dystrophies. Although Dr. Richard Sidman, Harvard University, has shown that there are parallels between human retinitis pigmentosa and hereditary retinal dystrophies of the rat, the similarity of these diseases is still speculative. However, studies of rats with retinal dystrophy are based upon the assumption that a better understanding of the animal condition will shed light on the human disease. Rats with dystrophic retinas have pigment epithelial cells which do not phagocytize debris from the retinal rod cells. This defect results in the death of these visual cells and blindness. Such a comparison of the normal and pathological changes that can occur in rats with and without retinal dystrophy aid our understanding of human retinal degeneration.

After the dystrophic rats are five weeks old, the production of outer segment material gradually ceases, the rod cells degenerate and the outer segment material is removed by the inner retina. The histologic appearance of the tissue when examined under an electron microscope is similar to that in the end stage of retinitis pigmentosa in man.

Dr. Liane Reif-Lehrer, Harvard University, has been studying the relationships of specific genes to retinal development in order to define the stages of development particularly vulnerable to the onset of retinal diseases. Such a study is also designed to provide a better understanding of the relationship between photoreceptors and the pigment epithelial cells and their interaction in retinal degenerations. Dr. Richard Sidman has found that "tetraparental" mice are created by combining two 8-cell stage mouse embryos to form one. The resulting mouse has, in effect, four parents--two from a normal strain and two afflicted with the hereditary retinal disease. In some members of a group of mice produced this way, patches of degenerated tissue were found in the retina interspersed among areas of partially affected or normal tissue. The proportion of normal to abnormal tissue in any individual rat appears to be directly correlated with the proportion of hemoglobin it has from each of the two strains. This "chimaerism" is believed to be the first demonstrated in mammalian nervous tissue. Dr. Sidman and his colleagues feel that this phenomenon will provide a useful tool for studying neural development and function by permitting the comparison of normal and diseased tissue in the same animal and as a model for sector retinitis pigmentosa. Visible light at intensities which are ordinarily encountered can be damaging to the retina and are being studied with NEI support. With the need for more knowledge of this phenomenon, the effects of light on the eyes of experimental animals are being studied by Dr. Melvin Rubin, University of Florida, and Dr. Werner Noell, University of Buffalo.

A basic phenomenon of physics is that light cannot be effective if it is not absorbed by a substance. In the retina, light is absorbed by the visual pigment called rhodopsin. When light is absorbed by rhodopsin, this complex molecule is rearranged as the sensation of light is initiated in the retina. The visual pigment must be resynthesized as the photoreceptors of the retina renew themselves. Studies in progress will identify and characterize the site of synthesis of visual pigment within the retina and will also reveal the nature of the nutrients (vitamin A) required for the synthesis of visual pigments.

Studies were designed by Drs. Dean Bok and Michael Hall, U.C.L.A., to determine at which stage of photoreceptor assembly, vitamin A derivatives are incorporated into visual pigment and whether they migrate to the pigment epithelium after visual pigment is bleached by light. It appears that exchanges between the outer segment of the photoreceptors and the pigment epithelium do not occur to any significant extent in frogs. Retinal (vitamin A) is added to opsin (protein) as new discs are synthesized at the base of the photoreceptor. Retinal does migrate to the pigment epithelium after release from rhodopsin in the albino rat, however, in pigmented rats, the situation may be comparable to that seen in the frog. Furthermore, in the rat, with an hereditary retinal dystrophy, the pigment epithelium does not phagocytize photoreceptors of the outer segment tips in a normal manner and death of the visual cells results.

Cage illumination from fluorescent light through a green filter leads to severe damage of the visual cells when continued for 40 hours. Vitamin A deficiency protects against this damage, but the products which result from the breakdown of rhodopsin are not the toxic substances. The normal diurnal cycle of light and dark may be the essential factor in controlling visual cell viability and susceptibility. Dr. Werner Noell, University of Buffalo, has shown that the light environment plays an important role in vitamin A deficiency and in the normal biology of visual cells and pigment eipthelium. These projects are providing new insights for the study of degenerative visual cell diseases.

Indirect ophthalmoscopy, histology and electroretinography show some similarities between human and rat retinal dystrophies. Studies on the effect of vitamin A deprivation on the rate of photoreceptor renewal will increase the understanding of retinal physiology and of previously untreatable diseases. The purpose of this study is to confirm the ability of vitamin deficiency to retard photoreceptor outer segment renewal and to establish means of monitoring this effect so as to obtain known quantities of photoreceptor renewal retardation without encountering photoreceptor cell death.

Retinal Tumors

Dr. Daniel Albert, Yale University, is attempting to learn more about pathogenesis and fine structure of pigmented tumors of the eye. The pigment epithelium and melanocytes are two populations of pigmented cells in the eye which differ in respect to their proliferative disorders, as shown in tissue culture. Retinal pigment epithelium exhibits non-neoplastic proliferation while uveal melanocytes show little tendency toward non-

neoplastic proliferation. Diagnosis of tumors of retinal pigment epithelium has caused difficulty because lesions representing the reactive proliferation of these cells often cannot be differentiated from true neoplasms. This study may help establish criteria for distinguishing between disorders that represent reactive proliferation and true neoplasms. These investigations may also provide information with regard to role of viruses in the etiology of ocular pigmented tumors, the malignant potential of pigmented cells, histology of reactive proliferation on neoplasia in retinal pigment epithelium, and histogenesis of malignant melanomas. The failure to find viral particles by electron microscopic examination is similar to results encountered in other human tissues, and, therefore, a study of reverse transcriptase in ocular tumors may indicate the presence of an oncogenic virus.

III. Disorders of Nervous Elements and Neural Pathways

This area of the program includes anatomical and neurophysiological studies of neural pathways from the retina to the lateral geniculate body and finally to the visual cortex of the brain. It also includes such topics as binocular vision, color vision and many psychophysical studies. This is a complex and little understood aspect in the field of visual research. A better understanding of the visual system between the retina and the brain is necessary for coping with diseases which affect vision but in which there is no apparent defect within the eye.

Dr. W. Maxwell Cowan, Washington University, has been studying some of the factors involved in the generation of specific neural pathways, and in particular the connections between the developing retina and the central visual structures upon which it projects. He achieved a significant technical advance necessary for the pursuit of this project by perfecting an autoradiographic method for tracing connections in the central nervous system. This method has already been adopted by a number of other senior investigators. Its chief advantages are that it is: (a) a highly repeatable; (b) based on a physiological property of neurons rather than on the induction of a series of pathological changes; (c) uncomplicated by interpretative difficulties of degeneration methods; and (d) readily quantifiable.

During the past year Dr. Cowan has investigated the sequence of changes which occur in the optic tectum of the chick after early removal of the optic vesicle or optic cup. Early deafferentation does not exert its effect upon cell proliferation or neuronal migration in the target zone, but mainly upon the further growth and maintenance of the cells which are normally in receipt of retinal fibers and, to a lesser degree, upon the second and third order neurons in the visual pathway. These effects have been documented in a large series of chick embryos. A second study deals with the rates of axoplasmic transport of proteins synthesized in the retinal ganglion cells, and their distribution to the various central nuclei upon which the retina projects. As in mammals, the transported proteins have been found to migrate at two velocities: a small amount preferentially distributed to the terminal portions of the axons; the bulk of the transported material becomes distributed along the whole length of the visual pathway. Auto-

radiographic studies have shown that this method of mapping the visual projection is as precise as any of the existing axonal degeneration techniques, and in addition it has the considerable advantage that it readily lends itself to tracing nerve connections.

Previously Dr. Cowan reported that during the normal development of certain visual centers, there is a progressive loss of cells. Since the importance of such morphogenic cell deaths in neural development is not generally recognized, Dr. Cowan and colleagues examined the fate of the large autoradiography and serial cell counts. When it is first formed, the mesencephalic nucleus of the chick contains about 4,000 cells; between the ninth and thirteenth days of incubation (when most muscle spindles are formed) the nucleus shows a dramatic cell loss amounting to about 75% of the total originally generated. The striking morphology, and the early development of this nucleus, makes it a particularly favorable model for this type of analysis. It is quite possible that comparable cell losses may occur during the development of all parts of the nervous system.

Dr. Lorrin Riggs, Brown University, is concerned with comparison of electrophysiological and psychophysical measures of visual function. For example, Dr. John B. Siegfried, formerly a student with Dr. Riggs, published a new method for determining the spectral sensitivity using the human visual evoked cortical potentials (VECP). The VECP is measured by placing electrodes on the scalp in the region of the visual cortex and recording the electrical potential at points relative to an electrode at a distant point. The response is largely from foveal stimulation. It has been used to investigate a variety of clinical problems, e.g. amblyopia, color vision, and visual acuity. Objective methods for determining spectral sensitivity for humans without requiring any judgment by the subject could have wide clinical applications.

It has been shown that individual cells in the visual system respond to specific stimuli such as vertical, horizontal and edges at various angles. These edges or lines are characterized by a sudden change in brightness. Dr. Russell De Valois, University of California, Berkeley, finds that single units in the monkey lateral geniculate nucleus respond to borders and to central regions of various types of visual figures presented to the eye when the figures are differentiated from their surroundings. Border enhancement was found in the response to luminance figures but not in the response to color figures. In addition, cells showed border enhancement only in the case of a figure which produced an increment in their firing rates. In situations in which very striking brightness contrast is seen perceptually, the cells do not show the corresponding changes in firing rate across the whole pattern. The lateral inhibitory mechanisms found in the retina and geniculate body can thus account for luminance border enhancement, but not entirely for simultaneous brightness or color contrast, for which other processes of some sort must be responsible.

Dr. Horace Barlow, University of California, Berkeley, is studying the fundamental aspects of vision from the initial events in the retina through the visual pathways and information processing to the mechanisms in the brain for perception. Drs. Barlow, Levick and Yoon are investigating the sensitivity threshold of retinal ganglion cell of the

cat. The purpose of this work is to measure the absolute sensitivity of cat retinal ganglion cells in order to learn how optic nerve impulses are related to absorption of light energy in the receptors. Statistical analysis of the results shows that: (a) up to 17% of the quanta entering the cornea must be utilized in the response; (b) as many as 3 extra impulses probably result from the absorption of a single quantum; and (c) the ratio of variance to mean of the pulse number distribution of the maintained discharge is similar to that of the response, and can be explained on the hypothesis of multiple impulses resulting from unitary events in the receptors.

Amblyopia

This disease may be defined as dull vision in the absence of ophthalmoscopically visible structural impairment. It may be classified on a functional basis into two groups: (1) functional amblyopia caused by strabismus or anisometropia, (both foveas receive images from the same object but the image in one eye is out of focus); and (2) amblyopia caused by lack of adequate retinal stimulation during the stage of visual immaturity, (amblyopia ex anopsia or stimulus deprivation amblyopia). One cause of this type, for example, is opacities of the ocular media (e.g. cataracts) at birth or soon thereafter.

Dr. Gunter Von Noorden, Johns Hopkins University, has been studying experimentally produced amblyopia with monkeys. The right eyes of rhesus monkeys were surgically closed at various ages in early life, and the animals were trained to respond to visual acuity testing. After the closed eyelids were reopened, the non-deprived eye was closed so that visual acuity of the deprived eye could be tested. The results show a direct relation between the age at which the lid was closed and visual impairment. The critical time at which foveal vision deprivation no longer interferes with subsequent visual recovery lies between the first and third months of life. The animals whose eyes were closed after the age of 3 months showed rapid and complete recovery to the highest tested level of acuity in the non-deprived eye. However, when lid closure was performed within the first 12 days of life, the animal was not only unable to recognize test patterns but also failed to respond to red. Lid closure at the age of 4 weeks similarly caused severe impairment of pattern vision but red light sensitivity was maintained. These results are in agreement with similar experiments published by Drs. David Hubel and Torsten Wiesel, Harvard University, on kittens which were deprived of pattern vision. The results of this study are of both theoretical and clinical interest. A model has been created in which the nature of stimulus deprivation amblyopia can be further studied. This is important because the exact age at which the human visual system is sensitive to form vision deprivation is unknown, even though clinical experience seems to limit it to the first 2 or 3 years of life.

Dr. Von Noorden also has reported on experimental investigations of strabismic amblyopia. He succeeded in producing strabismic amblyopia experimentally in rhesus monkeys which were surgically made esotropic (turned inward). At the ages of 1 and 7 days they develop amblyopia whereas those with onset of experimental strabismus at age 17 months do not. Exotropic strabismus did not result in amblyopia. Vision in the ambly-

opic eye improved slowly if the normal eye was surgically closed for a period of time. These results are in agreement with clinical experience with humans.

An electron microscopic study of synapases from areas 17 and 18 in normal and in the visual cortex from an amblyopic monkey was conducted. Synaptic density and morphological synaptic characteristics were described and compared. No significant difference existed in the number of synapses counted in comparable tissue from both specimens. Data collected for different parameters of synaptic characteristics (length of synaptic contact, distribution, number and shape of presynaptic vesicles and mitochondria) also show that no differences are present in the visual cortex of amblyopic and normal animals. These preliminary findings indicate that the severe neurophysiological defect demonstrated in the visual cortex of monkeys with strabismus and stimulus deprivation amblyopia does not have an analogous morphological equivalent in the visual cortex.

IV. Eye Movements and Their Disorders

In order for the eyes to function it is necessary for them to maintain fixation on a stationary object, follow moving objects and generally to survey the surrounding world. When a subject attempts to maintain fixation of a stationary object he brings its retinal image to fall upon a very circumscribed portion of his retina. He can maintain the retinal image of the target object in this preferred position a minute or more. During this period, very small rapid eye movements, known as saccades and low velocity eye movements known as drifts, occur. Some of these movements serve the purpose of returning the retinal image of the fixated object to the preferred position; others result from intrinsic instability in the oculomotor system. Actually some motion of the eyes is necessary in order to maintain vision. Other eye movements are convergence in which eyes rotate inward toward each other or divergence in which they rotate outward simultaneously. For following moving objects there are two types of conjugate eye movements, saccadic and smooth pursuit. Saccadic movements are made not only for fixation but also in response to step displacement of visual stimuli. The pathways by which visual inputs eventually reach the oculomotor nerves and results in eye movements are not well established. Direct projections to the oculomotor nuclei from visual cortex, frontal eye fields, pretectum and superior colliculus are not known. However, each of these areas has been implicated in eye movement control. The study of eye-movement control system has applications with respect to strabismus surgery and other clinical problems related to eye movements.

Strabismus (heterotropia, squint, wall eyes, or cross-eyes) is that condition in which an object in space is not imaged simultaneously on the fovea centralis of each eye. In this section of this report some of the recent results of grantees on the mechanism of eye movements and strabismus will be reviewed.

Dr. Barbara Wickelgren, University of Oregon, is investigating the way in which the colliculus processes visual, auditory and somatic information and the way in which the putput of the colliculus is used to control motor responses. Deep layer cells, like

superficial ones, respond well to moving line stimuli (slits, bars, edges, and tongues), are binocularly driven, require moving stimuli, and are usually directionally selective for stimuli moving toward the periphery of the visual field. The receptive fields of the deep cells do, however, differ from those of the superficial cells in a number of ways. Deep cells relate to very large receptive fields and sometimes include the entire part of the contralateral visual field covered by the tangent screen. The receptive field borders for deep cells are frequently vague and difficult to map; the responses decrease very gradually as the stimulus moves away from the most sensitive portion of the visual field. Deep cells also fire very erratically in response to repeated presentations of the same stimulus. This makes it extremely difficult to do quantitative studies on the precise properties of the optimal stimuli for these cells. Many deep cells responded to a wide range of stimulus sizes and shapes, and some cells respond equally well. Deep cells are more likely to show absolute directional selectivity; that is, complete failure to respond to stimuli moving in the null direction. Deep cells are also more likely to respond optimally to very rapid stimulus movement.

For a study of cortical cells, squint was produced artifically in 2-3 week old kittens by surgery. The effects of monocular eye surgery were similar regardless of the particular muscles cut. As reported by Drs. David Hubel and Torsten Wiesel, Harvard University, most visual cortical cells in all squint animals are monocularly driven. In squint animals 3-6 months old, most collicullar cells are binocularly driven, as they are in the normal animal. Only in squint animals one year old, does the ocular dominance histogram become abnormal. Over 84 percent of the cells in the colliculus contralateral to normal eye are dominated by this eye. In the colliculus contralateral to the operated eye, only 31 percent of the cells are dominated by the operated eye, 27 percent are dominated by the normal eye, and 42 percent are driven equally by both eyes. In contrast, the visual cortex shows only a slight predominance of the normal eye over the squint eye. The differences between the receptive fields of collicular and cortical cells and the different effects of strabismus on the two structures provide additional support for the notion that the cortex and the colliculus use sensory information which enables an animal to visually track a stimulus regardless of the modality with which that stimulus first impinges upon animal. These cells might control head, eye, or body movements. These studies are important in an understanding of eye movement pathology. Damage to extraocular muscles in young children may result in the loss of function in one eye. The present results show that damage to cortical function occurs much earlier in an animal's life than does damage to collicular function.

Dr. Peter Schiller, Massachusetts Institute of Technology, is studying the role of the superior colliculus. Single units in the superior colliculus of the alert monkey were recorded. In order to establish the nature of the visual input and its relationship to eye movements, one eye of each monkey was immobilized by transection of cranial nerves. The receptive field of single cells in the superior colliculus was mapped on a tangent screen facing the animal. Eye-movement-monitoring electrodes were implanted around the moving eye to study unit activity in relation to ocular motility. Superficial layers of the colliculus units have visual receptive fields. Responses show no specificity to

visual stimuli in terms of pattern or in terms of direction of movement, and both of these attributes are common in cells of visual cortex. In the colliculus there is specificity in terms of stimulus size; however, large stimuli are typically ineffective in eliciting spike activity. Responses in the superficial layers are best to smoothly moving stimuli. In the intermediate layers a gradual transformation can be observed. In this area the cells preferred rapid, jerky stimulus displacement; smooth movement is ineffective. In the lower layers of the colliculus, eye-movement cells predominated. These cells have a dual property. They have visual receptive fields, and they fire prior to saccadic eye movement. Specificity was observed in terms of the size and direction of the saccades with which a unit fires. This specificity for each unit corresponds to the location of the unit's receptive field relative to the fovea. Thus, it appears that the result of unit discharge is to position the fovea relative to stimuli which were presented in the receptive field of the unit in question. Discharges are independent of initial eye position in orbit. These findings suggest that the superior colliculus of the monkey plays a role in the acquisition of visual targets for foveal viewing by initiating saccadic eye movements.

Similar methods with the addition of electrical stimulation are being applied to the stimulation of superficial layers of the colliculus to produce saccades. The size and direction of the saccade is dependent on the site of stimulation and is relatively independent of stimulation parameters. Long trains elicit a staircase of saccades, where each saccade is of the same size with intervening fixations. A close correspondence exists between receptive field locations and the elicited saccades. Stimulation elicits a saccade which brings the fovea to that part of the visual field where the receptive fields of units stimulated were located. In the lower layers of the superior colliculus a close correspondence was found between unit activity and stimulation. These findings are in consonance with the view that the superior colliculus plays a role in eye centering.

Saccades may be involuntary (reflexive) to maintain fixation or voluntary to follow an object. Dr. Robert Steinman, University of Maryland, has been studying the voluntary versus the reflexive aspects of oculomotor behavior. Recent work by Dr. Steinman using equipment capable of recording smaller saccades with greater accuracy and reliability, has shown that human subjects when asked to track very small target steps make saccades as small as those used for fixation. This indicates that both types of saccades employ the same control mechanism and that all saccades can be brought under voluntary control.

Eye movement research is significant to health problems, because the human's ability to place stationary or moving details in the center of best vision and maintain it there is his most accomplished motor skill. Understanding the high and low velocity system that is used to control eye position is needed for the diagnosis and treatment of a number of maladies that affect the oculomotor system directly or in which oculomotor symptoms are frequently observed, e.g. squint and multiple sclerosis.

Dr. David Robinson, Johns Hopkins University, is investigating whether the relationship between motoneuron discharge rate and eye movement (especially eye velocity) is the same for visually-guided pursuit movements and for vestibular slow phases. Vestib-

ular slow phases were induced by rotating the subjects (monkeys). Recordings were made in the abduceus nucleus in the brain. The relationship between motoneuron discharge rate and eye movements was the same for pursuit and for vestibular-induced movements and thus, vestibular eye movements are not mediated by a special subset of motoneurons.

A neural network must exist between the vestibular ocular nucleus and the motoneurons which acts like an integrator. These findings pin down exactly the role of the common path in the vestibular ocular reflex. Whether or not the cerebellum plays a role in the control of eye movements, or even of eye position, is an unaswered question. Although clinical observations provide some support for such a role, there is no clear evidence of single unit activity in the cerebellum correlated with eye movement. Dr. Bert Zuber at the Rush-Presbyterian-St. Lukes Medical Center, Chicago, has been reinvestigating this question. During this year Dr. Zuber and colleagues have shown that the evoked potentials are present even after the cerebellum is neurally disconnected from the rest of the brain, and thus, information related to eye muscle stretch is not reflected in the evoked potentials recorded at cerebellar sites. It is likely that these potentials have their source in deeper central nervous system structures and are volume conducted to the cerebellum. Dr. Zuber has also been investigating the nature of the signal in a part of the brain responsible for saccadic eye movements. He induced nystagmus by cutting one of the vestibular nerves. The firing rate of cells in the nucleus of the abducens nerve was recorded simultaneous with recording of eye movements. There is a direct relationship between the recorded activity in the brain and eye movement.

Dr. John L. Downer, University of Washington, is investigating eye-hand coordination mechanisms using normal and "split brain" monkeys. The reaction time, using the cross combination of left eye-right hand or the uncrossed combination of right eye-right hand, is approximately equal. However, the number of errors made in using the uncrossed combination (right eye-right hand) is significantly greater than the crossed combination (left eye-right hand). This supports previous observations that the awkwardness in visual tracking behavior observed in split-brain monkeys when using the uncrossed combination of right eye-right hand, or left eye-left hand, results from the inability to control accurately the positioning of the hand when using only visual cues. It was found that split-brain monkeys do not learn visual discrimination tasks by imitation or observation, i.e., the animals were initially trained in the visual tracking apparatus when using only one eye. Upon reaching the established criterion for accurate performance, the closed eye was opened, and the animals were retested on visual tracking behavior, with both eyes open, over a period of 6 weeks. The behavior was the same as that seen when the animals were using only one eye. Therefore, both halves of the brain received an identical visual input from the visual stimuli. Following this, the formerly occluded eye was left open and the eye used in the initial training was closed. When the animals were placed in the training apparatus they appeared to be completely unaware of the visual stimuli. Over a period of 48 hours no responses were recorded. It then became necessary to train them to respond to the visual stimuli. The time taken to learn the tasks was approximately the same as in the initial training procedure. Subsequent performance was within normal limits indicating that no pathological changes

had occurred in the eye. The conclusion drawnfromthese observations is that although one hemisphere observes what the other hemisphere is doing, this does not contribute to learned responses in the "naive" hemisphere.

Dr. Arthur Jampolsky, Institute of Medical Sciences, San Francisco, has a broad experimental and clinical program studying normal and abnormal oculomotor physiology and related to quantitative understanding of the physiologic mechanisms underlying the precise control and coordination of binocular eye movements. Human extraocular muscle mechanics and innervation patterns, and the pathophysiology of clinical motility disorders are the principal areas of interest and study. These studies have made it possible to assess in a quantitative way the restrictions to ocular motility and the active agonist muscle force. This has greatly improved understanding and surgical success in strabismus cases dominated by various combinations of restriction on paralysis. These include Brown's syndrome, other abnormal elevator palsies, lateral rectus paralysis, and many strabismus cases which have had multiple operations where restriction dominates the situation. Basically, one grasps the eye and pulls it, measuring thereby the restriction to motility. To measure active force one holds the eye while the eye is attempting to move, thus measuring the isometric agonist muscle force. Several instruments have been devised for clinical and laboratory investigation use.

V. Devices for the Blind

Dr. Carter Collins, Smith Kettlewell Institute, is developing a light-weight, battery operated, fully portable device which will project in tactile form upon the trunk a fac - simile image of the visual scene registered by a television camera. The device consists of a minature television camera attached to a pair of eyeglasses and an array of electrotactile stimulators (vibratory) in contact with the abdomen and back. The scene viewed by the television camera is thus transferred to a pattern on the stimulators. It is claimed that with one-hour training the blind person is able to recognize 25 common objects, discriminate among individuals, decide where they are in the room, and describe their posture, movements and individual characteristics such as height, hair length and presence of glasses. At present this device has been tested under optimum conditions such as good contrast between the object and its background. More extensive testing is needed to evaluate its usefulness for conditions and problems normally encountered by the blind.

VI. Collaborative Studies

The goal of laboratory research is the application of newly acquired knowledge to human diseases. Due to the limitations of experimental models or their absence, the advanced stage of laboratory investigations, or the urgent needs of administering to patients, certain visual disorders require investigations in human subjects. The limited number of subjects who can be recruited from a single clinic enforce the need for pooling of data accumulated at multiple clinical centers. Collaborative studies are encouraged in those situations which require the use of a large clinical population and senior scientists who are willing to cooperate with regard to the uniformity of experimental approach and control as judged by coordinating and data processing centers.

The NEI is sponsoring three collaborative studies: (1) Collaborative Glaucoma Study; (2) Collaborative Retrolental Fibroplasia Study; and (3) Collaborative Diabetic Retinopathy Study.

Collaborative Glaucoma Study

Investigators at five clinics have been conducting a long-term study of the natural history of open-angle glaucoma. Various parameters involved in ocular tension and aqueous humor flow are being explored for predictive value. The subjects include relatives of glaucomatous patients and a control group of non-relatives who are examined annually with the standard techniques of tonometry, tonography, water-drinking test, ophthalmoscopy and visual field examinations. During the early phases of this study, there was a low incidence of glaucomatous field defects. However, there is now an increase in the occurrence of glaucomatous field defects. It may now be possible to follow a population of patients who have developed visual field loss with respect to pressure, facility of outflow and cup/disc ratio.

Collaborative Retrolental Fibroplasia Study

An excess of oxygen has been identified as a contributing factor to the largest cause of infant blindness. A reduction of oxygen therapy in premature nurseries has dramatically reduced the incidence of this retinopathy. The occurrence of pulmonary diseases has encouraged the reintroduction of oxygen therapy. That arterial oxygen tension which is associated with retinal damage is unknown; therefore, this study is being conducted at five clinical centers and a coordinating and reading center in order to determine safe arterial oxygen tension levels for the premature infant and in order to establish an ophthalmoscopic examination for retinal vasoconstriction. The correlation of vessel caliber with arterial pO_2 will provide a means of protecting the premature infant from excessive oxygen tension. At present, the data suggest an inverse relationship between infant mortality and incidence of the disease.

Collaborative Diabetic Retinopathy Study

A protocol has been designed for a cooperative study to evaluate the effects of photocoagulation on progressive diabetic retinopathy. In this form of treatment, a high energy beam of light is focused on a small spot in the retina. The intense concentration of light energy is sufficient to coagulate the tissue, and thereby, seal or obliterate small blood vessels. The primary objective of this multiclinical study is to determine which of the photocoagulation techniques available are of greatest clinical importance. The evaluation of xenon and argon-laser photocoagulators will require long-term observation of retinas exposed to treatment. The relationships of changes in retinopathy will be observed by use of stereographic fundus photography. In addition, the information will be available to describe the natural history of diabetic retinopathy and the prognosis of treated and untreated eyes for various types and stages of diabetic retinopathy. In order to accomplish these goals, a coordinating center, a fundus photography reading center and a sufficient number of clinical centers to recruit over 1800 clinical cases are involved.

WASHINGTON UNIVERSITY (NIH-NEI-71-2289)

Title: The Development of an Automated Biomedial Image Processor

Current Fund Allocation: $119,142

Objective: This project is designed to assist in the advancement of neuro-ophthalmological research and has supported the development of a completely computer controlled microscope to track and display the dendritic organization of neurons. Through the development of computer hardware and software, biological data can be collected and analyzed. The operational system permits the rapid computation of cells in nuclear areas and of axon diameters.

Progress to Date: During the first nine months of the contract, equipment has been obtained, algorithms and operating systems have been developed for rapid computation of cells in neural areas and axon diameters, and a computer controlled microscope to track and display the organization of neurons has been built.

Significance to NEI Programs and Biomedical Research: This extremely promising work should be of great assistance in advancing ophthalmologic and neurological research. It could decrease the time for doing many routine but time consuming calculations by 100 fold.

Proposed Course of Project: It is anticipated that a one year extension of this contract will permit the contractor to interface image processing systems to an electron microscope; perform automatic counting of silver grains in autoradiographs; conduct a morphological study of Golgi impregnated neurons; and document and disseminate results of this project.

BOSTON UNIVERSITY (NIH-NEI-71-2513)

Title: Development of Clinically Useful Methods of Estimating Retinal and Choroidal Blood Flow

Current Fund Allocation: $297,000

Objectives: The objective of this contract is the development of clinically useful methods of estimating the rate of effective or nutrient blood flow to the human retina and choroid from the retinal and choroidal circulatory system. Two types of instruments are being developed for measuring arteriole-venous oxygen differences in the retinal and choroidal blood vessels. The fundus is photographed through green and red filters, and microdensitometry is performed on the photographic films. This procedure yields an estimate of the oxygen saturation of hemoglobin in the particular blood vessel analyzed. The optical system will be further refined to compensate for variations in the intensity of the illuminating flash.

A choroidal oximeter which uses photoelectric measurement of light reflected from the choroid is being refined. It will enable the operator to scan and to measure reflected light from small regions of retinal arteries and veins. The output of this measurement will be processed and the arteriole-venous oxygen differences calculated.

Progress to Date: A double beam photographic retina reflectance photometer is in operation and is being refined. Preliminary models of a scanning oximeter have been constructed and tested and useful signals are being obtained. A theoretical study of the various modes of analyzing these data have been worked out and the results incorporated into the design of the instruments.

Significance to NEI Program and Biomedical Research: This contract is of major importance to the Institute's retinal disease program. If successful, the instruments developed will provide a much needed research and diagnostic tool applicable to a significant portion of blinding eye diseases.

Proposed Course of Project: It is estimated that extension of the current contract for one year will enable the contractor to successfully develop techniques for calculating retinal and choroidal blood flow.

WASHINGTON UNIVERSITY (NIH-NEI-71-2514)

Title: Evaluation of the Effectiveness of Diphenylhydantoin (DPH) in Reversal of Recent Glaucomatous Field Defects

Current Fund Allocation: $101,595

Objective: This clinical trial has as its purpose to determine the effect of DPH on early glaucomatous visual field loss. Patients with primary open-angle glaucoma are randomly assigned to a treatment or control group. The clinical protocol includes the normal testing and management of ocular hypertension.

Progress to Date: During the first nine months of this project progress has been made in acquiring patients and getting this clinical trial underway.

Significance to NEI Programs and Biomedical Research: This project is part of a major special emphasis program of the Institute - the improvement of the prevention diagnosis and treatment of glaucoma. The successful development of more effective means of drug therapy for glaucoma patients would represent a major breakthrough in the treatment of this serious visual disorder which is one of the major causes of blindness.

Proposed Course of Project: It is anticipated that the remaining patients required for the study can be acquired before July 1973, so that the project can be completed by July 1974.

YALE UNIVERSITY (NIH-NEI-71-2512)

Title: Development of Drugs Useful in the Treatment of Glaucoma and Their Evaluation Both in Animals and Man

Current Fund Allocation: $113,018

Objective: A systematic search for adrenergic drugs with potential use in the treatment of open-angle glaucoma is being conducted. Compounds which interfere with the re-uptake and binding of norepinephrine and epinephrine and those which interfere with enzymatic degradation of norepinephrine may enhance and prolong the action of these drugs. The problem is approached through the testing and screening of drugs in animal models.

Progress to Date: Significant accomplishments to date are:

(1) An isolation chamber technique has yielded consistent, reproducible results. Strikingly regular dose-response curves are reported for the principal adrenergic agents. A number of catecholamines have been studied and defined according to their relative mydriatic potency.

(2) MacKay-Marg tonometry has proved to be a satisfactory method for the observation of pressure effects in the living rabbit. A large number of drugs, administered topically or intravenously have been studied.

(3) Methods for assaying monamine oxidase and catechol-o-methyltransferase in rabbit uvea are being developed.

Significance to NEI Programs and Biomedical Research: This project is part of a major special emphasis program of the Institute - the improvement of the prevention, diagnosis and treatment of glaucoma. The successful development of more effective means of drug therapy for glaucoma patients would represent a major breakthrough in the treatment of this serious visual disorder which is a major cause of blindness.

Proposed Course of Project: It is anticipated that the contract will be extended for an additional year to permit evaluation of the most active compounds in vitro and in vivo. A second portion of the study will involve a systematic examination of patients who are being treated with drugs that inhibit monamine oxidases. An attempt will be made to correlate the effects of topical administration of some of these agents on intraocular pressure.

GEORGE WASHINGTON UNIVERSITY (NIH-NEI-71-2503)

Title: Chromosomal and Histologic Evaluation of Human Embryos

Current Fund Allocation: $83,972

Objective: The objective of this contract is to assess the impact of known genetic errors (chromosomal syndromes) and/or mutagenic exposure (chromosomal breakage) on the eye and associated CNS structures in early organogenesis.

Progress to Date: In embryonic specimens evaluated to date, considerable autolysis was found even though tissue cultures were successful. In addition, most chromosomal abnormalities showed the poorest likelihood of tissue preservation (intrauterine necrolysis), and intact embryo recovery.

Significance to NEI Program on Biomedical Research: The embryology of visual structures and effect of gestational exposure to implicated mutagens and teratogens is of major importance in the understanding of and possible treatment of genetic and congenital abnormalities of the visual system.

Proposed Course of Project: It is anticipated that this contract will be extended without additional funds for a three month period for the purpose of collecting additional material and concluding the study.